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EDITOR'S VIEWPOINT

Irwin, Leaver and Walsh (p. 166), in their continuing studies of long chain amines as surface protectors against enamel surface decalcification, found straight chain primary amines most effective. A pantadecylamine, synthesized in their laboratory, was most effective but not enough better than a commercially available tetradecylamine to make it worthy of substitution in clinical studies since the commercial product is much less costly. This series of studies uses a different approach than most others to the caries-dentifrice problem. It is worthy of careful consideration.

Buxbaum, Kohn, Proutt, and Oster (p. 173) report a positive correlation between glycoprotein content and caries from a study of 36 hamsters fed diets with different quantities of dietary sugar. It is suggested that enamel glycoprotein may supply nutrient materials for the bacteria involved in the process of dental caries. This study is suggestive but more extensive investigation is necessary before any definite conclusions are drawn.

Zwemer (p. 182) attacked the problem of the possible role of fluorine in inhibition of acid formation by oral bacteria using an in vitro experimental method. The method should prove useful for investigating other agents alleged to act by inhibiting the activity of oral bacteria. With topically applied fluorides an inhibition of acid production was observed but this effect was greatly decreased in 24 hours and disappeared in 7 days. Although this study, as others, suggests that inhibition of acid production by bacteria is not significant when fluorides are applied topically, the possibility of inhibition by daily ingestion of fluoridated waters cannot be completely discounted. Because sodium silicofluoride is being added to many communal water supplies, Feldman, Morkin, and Hodge (p. 192) considered the state of fluorides in such waters. At pH 5 or higher, all of the sodium silicofluoride (up to 16 ppm of F) is hydrolyzed to silicic acid, fluoride ion and hydrogen fluoride. Since HF is only a fractional portion of the total F, it is all absorbed into the blood stream as F ion. Very little of the F will be bound to calcium, but in hard water as much as 28 per cent may be bound to magnesium. This chemical study indicates that sodium silicofluoride is nontoxic in communal waters above pH 5 and below 16 ppm of F.

Studies on fluorine content of teeth and fluorine content of domestic waters show a positive relationship between each other while caries rates and fluorine show inverse relationships in Israel. This report of Gdalia and Yardeni (p. 203) confirms similar reports from other countries. The lack of increased uptake of fluorine in hot regions (with higher water consumption) may indicate a maximum level of metabolism from waters despite increased uptake.

Hill, Blayney, and Wolf (p. 208) give a progress report (number 15) on the Evanston Dental Caries Study. This fluoridation project was initiated in 1946 and fluorine added in 1947. The present report is based on examinations made up to 1954. The decrease in caries rates in 6- to 8-year-olds, after 71 to 82 months, was 58 per cent and in 12- to 14-year-olds, after 83 to 94 months, was 22 per cent. As in other areas, the fluoridation of water in Evanston is proving effective in controlling dental caries.

McClendon and Gershon-Cohen (p. 220) fed rats diets high in fluorides (tea or tea and camellia supplements). The high fluoride diets were cariostatic but at the high *tea* concentrations used, the high caffein, low caloric content resulted in death of the animals. It is suggested that dietary fluorine may retard caries in human beings if the concentration of fluorine is sufficiently high.

Following the demonstration of increased protease activity of salivary glands from

caries-susceptible rats compared with caries-resistant, Willett, Rosen, Stafseth, Hunt, and Hoppert (p. 223) investigated the protease activity of salivas from genetically resistant and susceptible rats. The protease activity was found to be much greater in mature susceptibles than in the mature resistants but the difference was not marked in young animals, i.e., those at the age when caries would be developing. The authors, very wisely, point out that this protease activity may or may not be related to caries activity. While a relationship can be theorized, it is also quite possible that the two factors, caries and protease activity, are independent traits related to the inbreeding.

A brief paper by Anders (p. 230) is presented to support a previous report (*J. D. Res.* 35: 753, 1956). As with many biologic tests, there is indicated a danger in single tests for diagnostic and investigative purposes.

Dreizen, Gilley, Mosny, and Spies (p. 233) were able to produce brown pigment in decalcified areas of teeth, similar to that in carious lesions, by exposing the organic matrix to degradation products of glucose. They suggest that the melanin-like pigment in human caries results from interaction between carbohydrates and proteins, although they accept the possibility that, in vivo, foodstuffs or bacteria may supply tyrosinase to induce brown pigmentation of carious lesions.

Shiere (p. 237) tested a tyrothricin-containing dentifrice in hamsters and in school children. The encouraging results in hamsters with a dentifrice containing 0.05 per cent tyrothricin with insoluble sodium metaphosphate and dicalcium phosphate as a polishing agent led to use of this formula in the school study. In 425 children of 7 to 13 years of age, divided into experimental and control groups, reductions of 35 per cent after one year and 26 per cent after two years were observed. The results are worthy of consideration in the search for an anticaries dentifrice.

Schuback and Goldman (p. 245) describe a method of perfusing an animal with a mercury-gelatin mass for study of vascularity. A radiograph of the gingival region illustrates the applicability of this method in periodontal studies.

Goldman (p. 249), in a series of photomicrographs, illustrates the transeptal fibers in health and disease. He proposes that new groups of fibers form by uniting of previously existing fibers rather than by formation of new fibers.

Turesky, Crowley, and Glickman (p. 255) investigated the sulfhydryl and disulfide groups in gingiva, both inflamed and normal. The sulfhydryls increased in epithelium proliferating in regions of inflammation and were reduced in regions of degeneration. While the position of the sulfhydryl and disulfide groups could be observed in the gingiva by the Barnett-Seligman technic, their role in gingival change was not apparent.

Freinberg and Lite (p. 260), from a study of 7 patients, suggest that ascorbic acid may greatly increase the serum concentration of tetracycline antibiotics. If this limited investigation is confirmed by more extensive study, it may be possible to greatly reduce the effective dose of such antibiotics by using adjunct ascorbic acid therapy.

The teeth have been used for anthropologic studies by many biologists and the variations of tooth pattern appear to be well established. Ludwig (p. 263) selected the mandibular second premolar for analysis of morphologic variation and inheritance. A study of dental casts from 452 individuals established a group pattern that could be used for reference. These traits were then studied in 17 monozygotic twin pairs and 12 dizygotic twin pairs and the concordance analyzed. The complete study suggests the possibility of determining "ethnic affinity" by analysis of dental traits, and that these dental traits have a basis in inheritance. A few studies of dental traits in isolated ethnic groups are now under way and may, like this investigation, add to basic knowledge of inheritance and to use of dental traits in anthropologic surveys.

Boyd (p. 274) and Boyd, Sims, and Osgood (p. 281), in a series of two papers, describe the use of radioactive isotopes to investigate dental tissues. They used radioactive iodine, calcium and sulfur. Each radioactive element contributes to the study, radioactive sulfur giving greater

surface detail. Different exposures can be used for details of different tissues (enamel, dentin, and cementum).

Neumann and DiSalvo (p. 286) have demonstrated that teeth are compressible under loads comparable to those encountered in chewing. They suggest that such compression probably alters molecular orientation, diffusion, and maturation. While it can be concluded that compression of teeth under loads of chewing may alter the strength of the dental tissues it has not been established which stresses alter it favorably and which are unfavorable. These data are interesting but do not show that chewing a substance of given hardness will increase the strength of teeth, or resistance to disease.

Petersen (p. 291), investigating the use of diaphragms for dental x-ray units, found that, in addition to increasing the intensity of radiation in air, diaphragms may add secondary radiation. Since this radiation is greater when the diaphragms are closer to the radiated object, it is recommended that diaphragms be placed as close to the source of the x-rays as possible.

Cooley and Haberman (p. 294) found positive bacteremia in 39 per cent of 51 control patients and in 14 per cent out of 49 premedicated patients (two 250 mg. Terramycin capsules 3 hours before surgery) following tooth extraction under local anesthetics. When more than 4 teeth were extracted at one operation, there was a higher incidence of bacteremia than when fewer than 4 were extracted. All strains of *Streptococcus viridans* and *Staphylococcus aureus* isolated were sensitive to Aureomycin, Chloromycetin, and Terramycin. This study suggests that these "broad spectrum" antibiotics may be of value in premedicating patients with subacute bacterial endocarditis.

Bixler and Muhler (p. 304) had presented data indicating that feeding desiccated thyroid and giving fluorine supplements in water may have a synergistic effect. They fed desiccated thyroid and injected sodium fluoride. Under these conditions, the fluoride was ineffective indicating that in the previous study the fluoride had produced its effect topically.

King, Manahan, and Russell (p. 307) describe a "screening test" for possible inhibitors of caries. Such tests serve a valuable function in determining compounds to be tested for clinical value but should not be permitted to become fetishes. It is highly probable that sodium fluoride would not rate well in such a test which is designed to evaluate compounds intended to inhibit caries *by a given route*, i.e., inhibition of acid production in plaques. This does not detract from the value of such a test but emphasizes the obligation of the investigator to evaluate results properly in their limited, though useful, proportions.

Pigman, Gilman, Powell, and Muntz (p. 314) studied the action of 9 single strains of bacteria on the tooth in vitro in the "artificial mouth." Eight of these resulted in tooth destruction resembling caries under conditions of the investigation. Different organisms appeared to have marked differences in their effect on different dental tissues. They agree in their conclusion, generally, with the concept of W. D. Miller that many organisms may be active agents in the carious process rather than with the narrower view that lactobacilli are the sole etiologic agents.

H. B. G. R.

International Association for Dental Research

The next General Meeting of the International Association for Dental Research will be held in Detroit, Michigan, in March, 1958. All abstracts of papers for presentation must be in the Secretary's hands by November 4, 1957. Details will be published later.

FURTHER STUDIES ON THE INFLUENCE OF SURFACE ACTIVE AGENTS ON DECALCIFICATION OF THE ENAMEL SURFACE

M. IRWIN, A. G. LEAVER, AND J. P. WALSH

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WALSH and Green[1] investigated the possibility of covering the enamel surface, previously wet with saliva, with an acid-resistant film. In preliminary experiments they showed that fats, oils, and hydrocarbons had very little effect. Eventually long-chain amines were tried and found to exert a marked effect in protecting the enamel surface against decalcification by a lactate buffer at pH 4.0.

It was found that the application of a 1 per cent solution of hexadecylamine in medicinal paraffin to teeth, previously wet with saliva, afforded a considerable degree of protection against the pH 4.0 buffer, when compared with a control group of untreated teeth wet with saliva. Protection was of the order of 70 per cent over a 2-hour period and 50 per cent after 24 hours' exposure.

The mechanism of the protective effect seems to be due to the long hydrophobic chain which is water repellent and is attached to the tooth surface by the amine group, which apparently has an affinity for calcium phosphate. Green and Walsh[2] continued their investigations by examining the protective effects of the individual members of the homologous series of aliphatic amines. They observed that the amines containing 10 or fewer carbon atoms showed little or no effect, while the most effective ones were tetradecylamine and hexadecylamine, the former being significantly more so than the latter.

Whereas the long-chain amines are insoluble in water, their salts with mineral and short chain organic acids are appreciably soluble, the acetates and lactates being particularly so. It was found that, after addition of the amine to the buffer solution in which it dissolved as the lactate salt, a tooth placed in the solution was afforded the same degree of protection as another tooth brushed with the oil solution of the amine before being placed in the same buffer. This finding was of paramount importance as it showed that the protective effect could be obtained without the presence of oil, though it was considered that the oil solution might be more effective in vivo for mechanical reasons.

Subsequently Nevin, Walsh, and King,[3] Irwin and Walsh,[4] and Irwin, Leaver, and Walsh[5] have described further work in this field culminating in the formulation of an emulsion type dentifrice containing tetradecylamine acetate as its active principle. This preparation is at present undergoing clinical trial.

Received for publication Dec. 10, 1955.

The purpose of this present investigation was to investigate the possibility of finding a surface active agent exhibiting a significantly higher protective efficiency than tetradecylamine.

MATERIAL AND METHODS

Earlier work had demonstrated that only cationic surface active agents afforded protection to the enamel surface exposed to acid buffers; anionic and nonionic agents were completely ineffective. It was therefore decided to examine certain primary, secondary, and tertiary amines as well as some quarternary ammonium salts and some diamines. The majority of the compounds examined were supplied by a manufacturer* under various proprietary names. These preparations usually contain about 90 per cent of the principal constituent together with 10 per cent of similar amines from the homologous series. In addition to these preparations, various compounds, synthesized by us in the laboratory, were also subjected to test.

It had been shown previously that the water soluble salts of long-chain amines afforded a similar degree of protection both to teeth and to calcium phosphate powder. This finding was of considerable importance as the addition of 1 per cent of amine to an acid buffer, while not affecting the pH, conferred a similar degree of protection on a tooth subsequently placed in the buffer, to that obtained by brushing the tooth with an oil solution of amine prior to placing in the buffer.

Owing to the difficulty of obtaining large numbers of suitable teeth it was decided to estimate the protective capacities of the various compounds using powdered calcium phosphate as "substrate," with the proviso that any compounds exhibiting values superior than or equal to that of tetradecylamine would be repeated using individual teeth as "substrate."

In the testing of each compound the following technic was used: 10 ml. of lactic acid/sodium lactate buffer at pH 4.0 (checked on the glass electrode meter) were placed in each of 10 flat-bottomed glass tubes, 3 cm. in diameter. Then 0.1 Gm. of the amine to be tested was added to each tube and the mouth of the tube was covered with either aluminum foil or "Parafilm."† The tubes were then transferred to an incubator at 37° C. and kept there for 1 hour. Several compounds were examined concurrently in this way together with a control group of 10 tubes each containing 10 ml. of the buffer to which 50 mg. of calcium phosphate was added.

After 1 hour in the incubator the tubes were removed, their contents transferred quickly to centrifuge tubes and centrifuged for 5 minutes at 2,000 r.p.m. The calcium phosphate was thrown down and the supernatant liquid poured into clean tubes.

The volume in each tube was made up to 10 ml. (some evaporation occurred during incubation), and 2 ml. aliquots from each tube were titrated with ethylene diamine tetracetic acid in alkaline solution using ammonium purpurate tablets as indicator.

*Armour and Co., Chicago, Ill.
†Schaar & Co., Chicago, Ill.

Compounds giving a comparable protective capacity to that of tetradecylamine were subsequently subjected to a further test using teeth instead of calcium phosphate. In this test the amine was applied as a 1 per cent solution in medicinal paraffin and not as an aqueous solution of amine lactate. Two compounds which could not be subjected to the preliminary test were examined only in the second experiment. Most of the teeth used in this experiment were upper second incisors and upper canines. They were cleaned with pumice and washed with boiling carbon tetrachloride. The roots were covered with sticky wax and the enamel of the crown left exposed. Each tooth was wet with saliva, then brushed with a solution of amine in medicinal paraffin and placed in 10 ml. of the pH 4.0 buffer. Groups of 10 were again used, together with a control group in which the teeth were placed in the buffer immediately after wetting with saliva. The tubes were incubated at 37° C. for 18 hours, after which the teeth were removed from the tube with forceps and dried, pending visual examination. The liquid remaining in the tube was made up to 10 ml., and 2 ml. aliquots from each were titrated as in the previous experiment.

RESULTS

The object of the investigation was to obtain values for the protective capacity of a number of compounds with a view to the possibility of finding a more effective agent than tetradecylamine. The individual values are given here but no statistical comparisons have been made, except in the case of 2 compounds which exhibited a similar protective capacity to that of tetradecylamine on both the preliminary test and when subsequently tested in oil solution.

The concept of "protection" was introduced by Walsh and Green.[1] We have come to refer to the "percentage protective capacity" of a compound for the purpose of comparison of results in this field.

The percentage protective capacity=

$$\frac{\text{mg. Ca dissolved in control} - \text{mg. Ca dissolved in test} \times 100}{\text{mg. Ca dissolved in control}}.$$

In practice it is unnecessary to actually calculate the amounts of calcium dissolved and for practical purposes the expression may be written.

$$\frac{\text{mean value of titers (control)} - \text{mean value of titers (test)} \times 100}{\text{mean value of titers (control)}}.$$

The values for "percentage protective capacities," referred to in the tables as "protection," were calculated in this way. In the tables both the proprietary name and either the chemical name of the chief constituent or its formula are given, while compounds synthesized by us are marked with asterisks.

TABLE I

PRELIMINARY TESTS WITH CALCIUM PHOSPHATE POWDER PRIMARY AMINES

PROPRIETARY NAME	CHEMICAL NAME OF CHIEF CONSTITUENT	NUMBER OF CARBON ATOMS	PROTECTION
Armeen 10 D	Decylamine	10	12
Armeen 12 D	Dodecylamine	12	43
Armeen 14 D	Tetradecylamine	14	74
	* Pentadecylamine	15	83
Armeen 16 D	Hexadecylamine	16	64
Armeen 18 D	Octadecylamine	18	29
	* Oleylamine	18	31
	* Diiodo-octadecylamine	18	29
	* Monofluoro-octadecylamine	18	35

It will be noticed that the commercial preparations all contain an even number of carbon atoms. This is due to the fact that they are synthesized from naturally occurring fatty acids by way of the nitrile which is reduced to the amine. In this process, hexadecylamine is produced from palmitic acid, the 16 carbon atoms of the acid remaining in the amine. When palmitic acid is treated with hydrazoic acid (Schmidt reaction) a carbon atom is lost in the form of carbon dioxide and pentadecylamine is produced.

TABLE II

PROPRIETARY NAME	CHEMICAL NAME OF CHIEF CONSTITUENT	NUMBER OF CARBON ATOMS	PROTECTION
	Methyl dodecylamine	13	29
Armeen DMS	Dimethylated unsaturated amines	(Chiefly) 20	Nil
Armeen DM 16	Dimethyl hexadecylamine	18	10
	Dimethyl tetradecylamine	16	18
	Cetyl trimethyl ammonium bromide	19	Nil

Diamines.—

The four commercial compounds examined have the formula $R.NHC_3H_6NH_2$ and are fatty diamines containing 1 primary and 1 secondary amine group. The fourth one, Duomeen T di-oleate was actually tested in oil solution at 3 per cent concentration, using teeth and not calcium phosphate, but it is included in these results for the sake of continuity. The same applies to the sixth compound heptamethylene diamine di-oleate.

TABLE III

PROPRIETARY NAME	CHEMICAL NAME OF CHIEF CONSTITUENT	NUMBER OF CARBON ATOMS	PROTECTION
Duomeen 12	$C_{12}H_{25}NHC_3H_6NH_2$	15	58
Duomeen C	Similar C_{8-18} diamines	11-21	34
Duomeen T	Similar C_{16}-C_{18} diamines	19-21	24
Duomeen TDO	Di-oleate salt of above		
	(3 p.c. sol.)	19-21	28
	(0.5 p.c. sol.)	19-21 + (36)	8
	*Heptamethylene diamine	7	10
	*Heptamethylene diamine di-oleate	7 + (36)	10

*Compounds synthesized by us.

It is clear from the results of the preliminary tests that pentadecylamine was the only compound exhibiting greater protective capacity than tetradecylamine and that hexadecylamine and Duomeen 12 were the only others to approach it. These 4 were the only ones giving results exceeding 45 per cent. Accordingly these 4 compounds were examined in a further experiment in which their 1 per cent solutions in medicinal paraffin oil were applied to the exposed enamel of waxed teeth wet with saliva. The teeth thus treated were subjected to the buffer and the evaluation of protection carried out in the way previously described.

TABLE IV

PROPRIETARY NAME	CHEMICAL NAME OF CHIEF CONSTITUENT	NUMBER OF CARBON ATOMS	PROTECTION
Duomeen 12	$C_{12}H_{25}NHC_3H_6NH_2$	15	58
Armeen 14 D	Tetradecylamine $C_{14}H_{29}NH_2$	14	87
	Pentadecylamine $C_{15}H_{31}NH_2$	15	92
Armeen 16 D	Hexadecylamine $C_{16}H_{33}NH_2$	16	79

On examination of the individual titration values in each group it was found that the protection efficiency of Duomeen 12 was significantly less than those of the other 3.

Two statistical analyses were carried out using the "T" test. The protective capacity of tetradecylamine was shown to be significantly greater than that of hexadecylamine at 0.2 per cent level of probability. When the results of tetradecylamine and pentadecylamine were compared, it was found that the protective capacity of the latter was significantly greater than that of the former but only at 2.0 per cent level of probability.

In considering this experimental work it must be borne in mind that the tests using teeth give values which are suitable for comparative work but which are not absolute. In different series of tests the absolute values of protective capacities may vary somewhat but the relative effects of the different protective agents always bear the same ratio to one another.

Irwin, Leaver, and Walsh[5] have described the formulation of an emulsion type dentifrice containing 1 per cent of tetradecylamine acetate together with 0.5 per cent Duomeen TDO. The protective capacity of this latter compound has been shown to be 28 per cent at a concentration of 3 per cent (see above results) but to be less than 10 per cent (barely significant protection) when present in the emulsion at 0.5 per cent level. It is used merely as an emulsion stabilizer.

Two emulsions conforming to the formulation of Irwin, Leaver, and Walsh were prepared and their protective capacities estimated in the usual way using the exposed enamel surface of waxed teeth. The emulsions were of identical composition except that one contained tetradecylamine acetate as "active principle" while the other contained the acetate of pentadecylamine. The values obtained were as follows:

pentadecylamine	64 per cent
tetradecylamine	57 per cent

The individual titrations were examined and the 2 groups of results compared using the "T" test. Once again the protective capacity of pentadecylamine was significantly greater than that of tetradecylamine at the 2 per cent level.

DISCUSSION

The sets of results obtained for the majority of compounds giving relatively low values in the preliminary tests of protection (using calcium phosphate powder) were not subjected to statistical analysis and, consequently, only an over-all comment on the more obvious features is given here.

From the compounds studied it is clear that primary amines are very much more effective than secondary and tertiary ones while the quaternary compounds studied gave no protection whatsoever. The single secondary amine, methyl dodecylamine, gave a value of 29 as compared with 43 for dodecylamine itself. Of 3 tertiary amines tried, only dimethyl tetradecylamine gave any protection and that only 18 per cent, compared to the 74 per cent of the parent compound.

From the results obtained for four 18-carbon compounds, the saturated compound octadecylamine, the unsaturated oleylamine and 2 containing substituent halogens, it is clear that substitution of halogens or the presence of an olefinic linkage has little marked effect on the protective capacity of paraffin amines. However, it is possible that the low solubility of the amines of 18 and more carbon atoms might mask any significant differences. It is planned to prepare amines with substituent groups in the chain from compounds in the 14- to 16-carbon range.

The Duomeens are proprietary products of the formula $R.NHC_3H_6NH_2$ and thus possess both a primary and a secondary amine group. The results again indicate the paramount importance of chain length as only Duomeen 12, in which the total number of carbon atoms is 15, exhibits a protection of over 50 per cent. What is of peculiar interest is the finding, made abundantly clear in the subsequent work using teeth, that the straight chain pentadecylamine is very much more effective than Duomeen 12 which has the same chain length and an extra secondary amine group. The supreme position occupied by the straight chain compounds suggests that any break in the carbon chain by definite chemical linkage would result in diminished efficiency. The substitution of a hydrogen atom by a halogen, however, does not break the continuity of the carbon chain and, as mentioned before, it is intended to prepare amines with mono-halogen substitution, in the aliphatic chain.

The original finding of Green and Walsh[2] that the protective capacity varies with chain length in such a way that the maximum is reached in the case of the 14-carbon compound, with the 16-carbon compound slightly but significantly less, has been substantiated. The 15-carbon amine has been shown to give a higher value than the 14 in three different tests. Statistical analysis shows this to be significant though not at a high level. It is possible that the small increase is due to the fact that the pentadecylamine, synthesized

by us from redistilled palmitic acid, approaches 100 per cent purity whereas the proprietary compound Armeen 14 D contains 90 per cent tetradecylamine and 10 per cent of mixed dodecyl, hexadecyl, and octadecyl amines.

The emulsion dentifrice previously referred to is at present undergoing a clinical trial, the results of which will not be available until early in 1957. On the face of the in vitro tests, it does not seem necessary to replace tetradecylamine by pentadecylamine as the active principle of the emulsion. Not only is the increase in protective capacity small, but the method of synthesis is costly and would preclude any large scale development, particularly as the readily available Armeen 14 D is almost as effective. Apart from the one synthesis previously mentioned, work is now being confined to a comparison of the in vitro effectiveness of emulsion types of dentifrice containing 1 per cent of tetradecylamine but varying slightly in their formulation.

SUMMARY

1. The investigation of the effectiveness of surface active agents in inhibiting calcium dissolution has been continued.

2. In a preliminary experiment some 20 compounds were tested for their ability to reduce the dissolution of calcium phosphate by an acid buffer at pH 4.0.

3. Four of the compounds were tested for their ability to protect the enamel surface against the solvent action of the same buffer.

4. It was found that the straight chain primary amines were the most effective agents of those tested. Previous results indicating that tetradecylamine was the most successful of the aliphatic amines available commercially were confirmed.

5. It was found that pentadecylamine, synthesized in this laboratory, exhibited a slightly higher protective capacity than the commercial tetradecylamine, whether as the water soluble acetate, in oil solution, or in an emulsion dentifrice.

The authors are indebted to Armour and Co. of Chicago for gifts of several of their products. The work was carried out under a grant from the Medical Research Council of New Zealand.

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THE EFFECT OF DIET ON THE DEPOSITION OF GLYCOPROTEIN IN THE TEETH AND ITS RELATIONSHIP TO DENTAL CARIES IN THE SYRIAN HAMSTER

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INTRODUCTION

DIETARY influence on dental caries has been under investigation since Murphy's work in the early part of the nineteenth century.[1] The major portion of the research from 1800 until today, however, has centered about the extrinsic effects of diet on the mature erupted tooth.

The first major deviation in this philosophy occurred in 1950. Sognnaes reported that rats, hamsters, and monkeys did not develop appreciable amounts of caries in adulthood if their dietary carbohydrate was restricted during the period of pre-eruption of the teeth, even if the animals were fed high carbohydrate diets in adult life.[2] Sognnaes also reported that the reduction in caries in the teeth of European children during and after World War II did not parallel periods of reduced sugar consumption. Rather, those children whose teeth developed during periods of low carbohydrate intake showed a marked reduction in caries even after the sugar content of the diet returned to normal.[2]

It should be pointed out, however, that many variables were involved in Sognnaes' investigation, a pilot study in this field. His results, therefore, though very significant, are not conclusive. An excellent summary of this study and other similar ones have been thoroughly discussed by Toverud and associates.[3]

Although many investigators had used dietary changes to produce caries experimentally for a considerable period preceding 1950, the above two investigations were the first to demonstrate an intrinsic predisposing effect of diet on the teeth.

It is the purpose of the authors to further the investigation along this latter line of reasoning. Recent histochemical and electron microscopic studies of enamel have revealed the presence of glycoprotein in the matrix. An attempt will be made to correlate dietary content of carbohydrate with the deposition of glycoprotein and to compare the amount of this deposition with the incidence of dental caries in the hamster.

Data from thesis submitted to the faculty of the School of Dentistry of the University of Maryland in partial fulfillment of the requirements for the degree of Doctor of Dental Surgery.

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PROCEDURE

A colony of Syrian hamsters was raised and divided into 3 groups, each group being placed on a different diet (sugar-rich, sugar-deficient, and control). The constituents of these diets are tabulated in Tables I, II, and III.

TABLE I

SUGAR-RICH DIET

SUBSTANCE	MARYLAND
Sucrose	63%
Casein	26%
Corn oil	5% (to mix)
Yeast and liver extract	2%
Liver, concentrated salt mixture	Supplement
Salt Mixture	
Potassium	5.0 mg.
Calcium	145 mg.
Sodium	0.5%
Phosphorous	110 mg.
Magnesium	1.0 mg.
Chloride	0.5%
Iron	10 mg.
Manganese	1.0 mg.
Copper	1.0 mg.
Zinc	0.5 mg.
Iodide	0.5 mg.
Fluoride	0.1 mg.
Boron	0.1 mg.
Vitamins	
Choline	100 mg.
Inositol	50 mg.
Niacin	15 mg.
Pantothenic acid	5.0 mg.
Thiamin	5.0 mg.
Riboflavin	5.0 mg.
Pyridoxine	0.5 mg.
Folic acid	1.0 mg.
Vitamin A_1	5,000 U.S.P. units
Viosterol	500 U.S.P. units/Gm.
Vitamin B_{12}	1.0 mg.
Ascorbic acid	50.0 mg.
Vitamin E	10.0 units
Rutin	25.0 mg.

An attempt was made to raise 3 generations of animals within each of the dietary groups. This was not possible to achieve in some cases as later data will demonstrate. When each animal reached an age of 100 days, it was sacrificed; the mandibles and maxillae were dissected and preserved in 10 per cent formalin for approximately 2 weeks. At the end of this time, they were removed from the formalin, washed in warm water, and placed in small open beakers to dry for 2 weeks.

After this dissection, the jaws and teeth were then cleaned of all organic debris in preparation for the caries counts. The carious lesions were counted and recorded, following the technic of Keyes.[4]

Following the caries counts, the molar teeth were removed with small straight-nosed bone rongeurs; the incisors were picked free from the encasing bone. All available tooth structure for each animal was weighed accurately

TABLE II

CONTROL DIET (PURINA CHOW PELLETS)

Protein		26.8%
Arginine	4.3%	
Glutamic acid	16 %	
Glycine	5.4%	
Histidine	2.4%	
Leusine	8.2%	
Lycine	5.6%	
Methionine	1.7%	
Phenylalinine	4.6%	
Tryptophane	1.3%	
Tyrosine	4.2%	
Valine	5.4%	
Fat		5.63%
Fiber		4.44%
N.F.E.*		46.46%
Calcium		1.38%
Phosphorous		0.96%
Magnesium		0.17%
Iron		326 ppm
Manganese		91 ppm
Copper		12.9 ppm
Cobalt		0.12 ppm
Iodine		5.20 ppm
Potassium		0.6 %
NaCl		0.97%
Carotene		6.0 ppm
Thiamine		14.7 ppm
Riboflavin		7.4 ppm
Niacine		66 ppm
Vitamin D		4.97 units/Gm.
Vitamin A		9.20 international units/Gm.
Pantothenic acid		15 ppm
Choline		1.70 ppm

*N.F.E. stands for nitrogen-free extract, and represents the carbohydrate portion of the diet. It is primarily composed of starches.

TABLE III

DIET COMPARISONS

INGREDIENT	SUGAR DEFICIENT (%)	SUGAR RICH (%)
Powdered whole milk	70	35
Alfalfa meal	29	13
Sucrose	—	51
Salt	1	1
Geveral	2	2
Oil (corn)	8	8
Total carbohydrate (H_2O)	35.6	63

(to the fourth decimal place) before and after pulverization. Mortar and pestle proved to be the most satisfactory method of performing the pulverization. The grindings were then stored in small ground glass-stoppered bottles.

The reagent employed for the determinations of glycoprotein in tooth structure was anthrone. It was selected not only because of its specificity in polysaccharide determinations, but also for the relatively simple manner in which the tooth structure could be handled when employing this reaction.

The anthrone was made up in a 1 per cent solution with fresh, colorless reagent grade ethyl acetate. This solution could be utilized for a maximum of 3 days when stored at 4° C.

The stock sugar solution from which the standards were made was an equimolar solution of galactose and mannose (295 mg. of galactose and 205 mg. of mannose in a liter of physiological [0.85 per cent] saline). The concentration of the stock solution was 0.5 mg. per cubic centimeter. The appropriate stock dilutions were made to achieve standards over a concentration range of 0.010, 0.025, 0.037, 0.050, 0.075, 0.10, 0.125, 0.150, 0.175 mg. per cubic centimeter with physiological saline used as diluent.

The standard run was set up by designating a pair of Folin tubes for each dilution. Three cubic centimeters of the standard was added to each tube, and another pair of tubes retained as control blanks contained only physiological saline. Into each of these tubes 0.5 c.c. of the anthrone reagent was pipetted. Then 5.0 c.c. of 95 per cent clear sulfuric acid was slowly layered

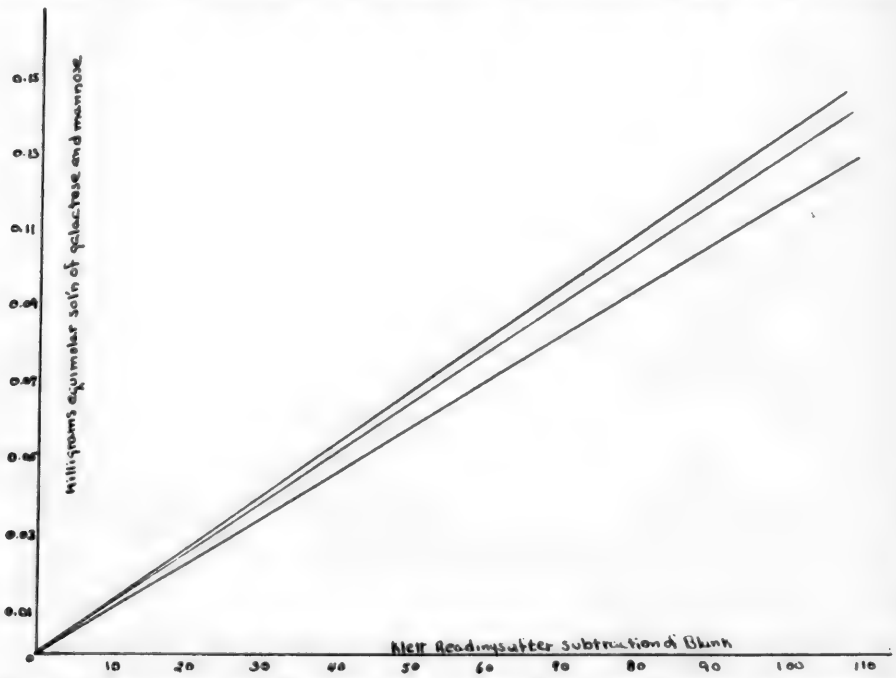

Fig. 1.—Standard curves of Klett readings determined from known polysaccharide concentrations.

onto the mixture and mixed by gently swirling the tubes and slowly accelerating the speed of the swirling operation. If the swirling was stopped before the thorough mixing occurred flocculation resulted and the tube in question had to be discarded. Color developed almost immediately and increased in intensity for the first 15 to 20 minutes. The optimum time for reading the tubes was found to be 50 minutes after acid addition. At this time, the color intensity was most stable.

The solutions in the Folin tubes were transferred to colorimeter tubes for reading. These tubes had been previously calibrated and were only used

when the optical density varied less than 4 points on the Klett scale. The colorimeter tubes were placed in the Klett colorimeter and the readings were taken directly from the scale of the machine.

The standard runs were repeated many times until there was no doubt as to the reproducibility of the standard curves. These runs established standard light transmission curves on the basis of specific Klett readings for standard polysaccharide solutions, of known concentration (Fig. 1).

When actual tooth structure was analyzed for the polysaccharide of the glycoprotein moiety, physiological saline was added to the grindings of the teeth of these animals in the ratio of 1 c.c. per 30 mg. of tooth structure. This mixture was left in the refrigerator for 24 hours for a "gentle" extraction of the glycoprotein. Refrigeration prevents excessive bacterial decomposition of the glycoprotein. The material was then centrifuged for 10 minutes at 3,000 r.p.m. and the supernatant fluid containing the glycoprotein was saved. Physiological saline was used for the extraction of the glycoprotein because there is less possibility of denaturing the protein moiety when using this solution. The residue from the extraction is chiefly tricalcium phosphate, which was examined electrophoretically for the presence of glycoprotein. Since this test was negative, we can assume that the glycoprotein was extracted to a negligible residue by the saline.

The residue and supernatant liquid from a sugar-rich tooth sample were also examined for the presence of glucose, by the use of Benedict's qualitative reagent. An analysis made for glucosamine (after deamination) on tooth extract by the use of the indole reaction indicated the presence of a relatively small amount of hexosamine. Therefore, we can safely assume that there is no free glucose and very little glucosamine present in the tooth extractant.[5]

From the saline extract, 2 portions (3 c.c. each) were taken for the quantitative determinations. This procedure was standard with all grindings except for sugar-rich animals, for which 2 portions (1.5 c.c. each) were used to obtain readable results. The glycoprotein in solution was next precipitated by the action of 10 c.c. of absolute alcohol for 4 hours. The precipitate was centrifuged for 10 minutes at 3,000 r.p.m. and the supernatant fluid poured off. The precipitate was washed with 10 c.c. of absolute alcohol and centrifuged again for 10 minutes at 3,000 r.p.m. The supernatant fluid was poured off carefully, and 3 c.c. of physiological saline was added to dissolve the precipitated glycoprotein. The dissolved glycoprotein was transferred to Folin tubes, and anthrone and acid were added as described previously. The Klett readings were all performed with the same technic as the standard runs.

RESULTS AND CONCLUSIONS

From the standard polysaccharide curve, the Klett readings of the unknown tooth samples were read in milligrams of glycoprotein per 3 c.c. of saline for control and sugar-deficient animals and milligrams of glycoprotein per 1.5 c.c. of saline for sugar-rich animals. The alteration in technic was necessary because of the excessively high Klett readings obtained when 3 c.c. extracts of sugar-rich tooth structure were used. At least 3 standards were

employed in every run to ascertain which standard curve was being duplicated. Reproducibility of the standard curve was possible within ±10.5 per cent error.

Tables IV, V, and VI summarize the hamster generation, caries score, powdered weight of tooth structure in milligrams, milliliters of saline added, Klett reading, milligrams of glycoprotein per 3 c.c. (and 1.5 c.c.) of saline,

TABLE IV

SUMMARY OF RESULTS OF SUGAR-DEFICIENT GROUP

GENERATION*	ANIMAL	CARIES SCORE	POWDERED WT. OF TOOTH STRUCTURE IN GM.	MILLILITER OF SALINE ADDED	AVERAGE KLETT READING MINUS BLANK	MG. GLYPR./3 C.C. OF SALINE	MG. GLYPR./GM. OF TOOTH STRUCTURE
First	G_1F_{19}	0	0.2393	7.9	0.25	0.010	0.110
First	G_1M_{15}	1	0.0458	3.0	3.50	0.005	0.109
First	G_1F_{20}	4	0.2810	9.3	4.12	0.054	0.250
First	G_1F_6	2	0.2023	6.7	3.25	0.004	0.044
First	G_1F_{13}	3	0.1921	6.4	9.0	0.012	0.133
First	G_1M_4	0	0.2702	9.0	11.0	0.014	0.155
First	G_1M_{17}	1	0.0461	3.1	10.0	0.012	0.132

*Due to the nutritional problems encountered with the sugar-deficient strain of hamsters, the successful rearing of mature second and third generation animals was not accomplished.

TABLE V

SUMMARY OF RESULTS OF CONTROL GROUP

GENERATION	ANIMAL	CARIES SCORE	POWDERED WT. OF TOOTH STRUCTURE IN GM.	MILLILITER OF SALINE ADDED	AVERAGE KLETT READING MINUS BLANK	MG. GLYPR./3 C.C. OF SALINE	MG. GLYPR./GM. OF TOOTH STRUCTURE
First	G_1M_3	1	0.1535	5.1	19.5	0.028	0.310
First	G_1M_1	1	0.1898	6.3	10.5	0.0135	0.148
First	G_1M_2	3	0.2691	8.9	26.5	0.037	0.310
First	G_1F_8	4.5	0.2049	6.8	23.5	0.035	0.308
Second	G_2F_8	2	0.1972	6.5	10.0	0.016	0.176
Second	G_2M_5	3	0.0791	2.6	14.5	0.017	0.216
Second	G_2M_6	3	0.1286	4.2	19.5	0.027	0.294
Second	G_2F_{11}	5	0.3301	11.0	12.5	0.015	0.167
Second	G_2M_8	4	0.2901	3.0	9.0	0.012	0.079
Second	G_2F_9	1	0.1082	3.6	9.0	0.012	0.042
Third	G_3F_1	2	0.2052	6.8	23.0	0.022	0.243
Third	G_3M_1	4	0.2680	8.5	25.1	0.025	0.277
Third	G_3F_2	3	0.2795	9.3	19.5	0.027	0.299
Third	G_3F_2	3	0.2965	9.8	21.0	0.029	0.319

and milligrams of glycoprotein per gram of tooth structure for every animal tested in the different diet groups. Results are averaged in tabular form in Table VII. In many cases the number of carious lesions increased in direct proportion to the increase of glycoprotein. It should be noted that there was little difference in results between sugar-deficient and control groups. This observation is entirely to be expected since there was only a 10.86 per cent carbohydrate difference between the 2 diets. However, the effect of diet is quite vividly demonstrated when the sugar-rich group is analyzed.

The sugar-rich group of animals was maintained on a diet containing 16.54 per cent more carbohydrate than the control group and 35.6 per cent more carbohydrate than the sugar-deficient group. The increased dietary

TABLE VI

SUMMARY OF RESULTS OF SUGAR-RICH GROUP

GENERATION	ANIMAL	CARIES SCORE	POWDERED WT. OF TOOTH STRUCTURE IN GM.	MILLILITER OF SALINE ADDED	KLETT READING	MG. GLYPR./1.5 C.C. OF SALINE	MG. GLYPR./GM. OF TOOTH STRUCTURE
First	G_1F_{31}	12	0.3122	8.4	21.0	0.030	0.538
First	G_1F_{23}	14	0.2367	7.8	12.0	0.018	0.311
First	G_1F_{30}	24	0.1059	3.5	15.0	0.018	0.403
First	G_1F_{15}	46.5	0.2302	7.6	58.5	0.080	1.760
First	G_1F_7	25.5	0.4430	14.7	21.2	0.032	0.708
First	G_1F_{32}	19.5	0.1925	6.4	21.5	0.032	0.709
First	G_1M_{16}	27	0.1756	5.8	24.7	0.034	0.734
First	G_1F_{24}	34.5	0.2429	8.1	15.0	0.018	0.402
First	G_1F_{21}	26	0.1457	4.8	22.0	0.031	0.651
First	G_1F_{28}	26	0.1769	5.8	21.0	0.030	0.656
First	G_1M_{18}	27	0.2057	6.8	24.0	0.034	0.703
First	G_1F_2	26	0.2160	7.2	22.5	0.032	0.711
First	G_1F_{22}	26	0.2367	7.8	12.0	0.023	0.503
Second	G_2F_2	27	0.5675	18.6	16.5	0.030	0.724
Second	G_2F_3	45	0.1063	3.5	23.0	0.042	0.923

TABLE VII

SUMMARY OF AVERAGE RESULTS

GENERATION	DIET	NUMBER OF ANIMALS IN GROUP	AVERAGE CARIES SCORE	AVERAGE MG. GLYCOPR./GM. OF TOOTH STRUCTURE
1	S. D.	7	1.57	0.133
1	Control	4	2.37	0.269
2	Control	6	3.0	0.162
3	Control	4	3.0	0.284
Average of all controls		14	2.79	0.283
1	S. R.	13	23.3	0.599
2	S. R.	2	36.0	0.8235
Average of all S. R.		15	29.65	0.711

carbohydrate of the sugar-rich group became manifest in the comparative caries scores and glycoprotein content of the hamster teeth. The sugar-rich animals exhibited caries scores which were 18.8 times greater than the sugar-deficient group, and 10.6 times greater than the control group. Glycoprotein results were similar. Sugar-rich animals showed an average of 585 mg. per cent more dental glycoprotein* than sugar-deficient animals and 438 mg. per cent more than the control animals.

Variations from the direct relationship between caries incidence and glycoprotein deposition were noted among some animals of the same dietary group. However, this variation may be due to any number of uncontrolled factors in the individuality of the animals or in the technic of measurement. Tooth morphology, hypocalcification, specificity of the anthrone reaction, and temperature of the reaction may easily account for this variability. The basic pattern of the caries-glycoprotein relationship is, however, consistently uniform.

*The colorimetric readings for polysaccharide values are considered as representative of the glycoprotein compounds hence are referred to as glycoprotein concentrations.

The relationship between caries susceptibility and dental glycoprotein has been demonstrated graphically in Fig. 2. Here it is shown that caries susceptibility in the sugar-rich animals is significantly higher than either the sugar-deficient or the control animals and that this susceptibility is directly proportional to the quantity of glycoprotein found in the teeth. The curves plotted in Fig. 2 demonstrate graphically the correlation between the two factors as all three slopes approach the diagonal. The differences between the slopes of the sugar-rich and sugar-deficient diet curves compared to the control diet curve demonstrate statistically significant differences. These differences, as given in Table VIII, are far in excess of the required magnitude for significance, that is, 3 times the standard error.[6]

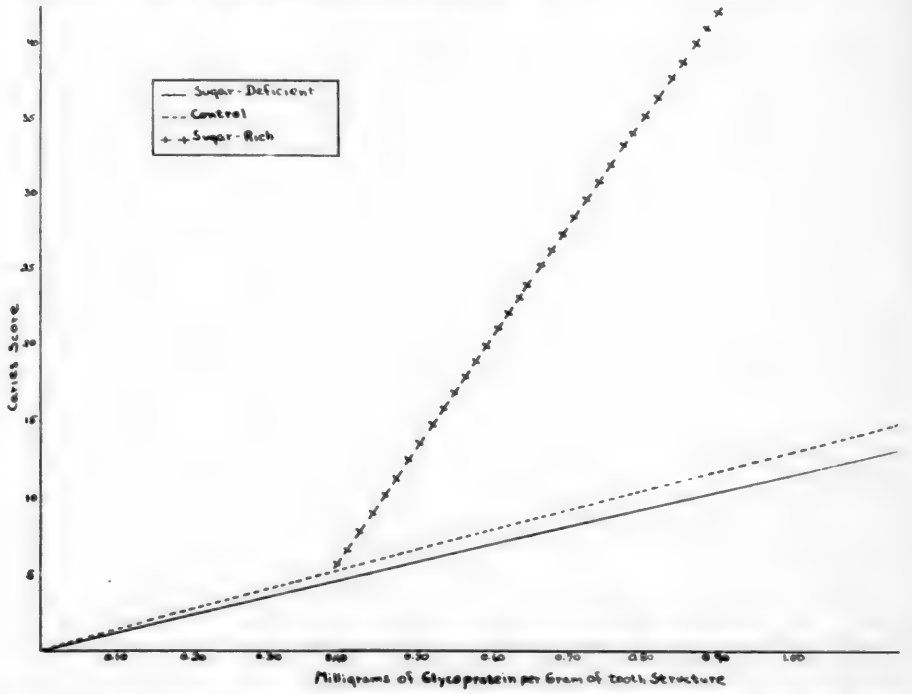

Fig. 2.—Relationship between caries susceptibility and dental glycoprotein in the Syrian hamster.

Since there is such a dramatic increase in the dental glycoprotein of the sugar-rich animals, these hypotheses may be suggested: all other factors being equal, first, the caries susceptibility of a tooth is directly proportional to the glycoprotein content; and second, the deposition of this substance in the tooth is greatly influenced by the nutrition of the animal during the period of tooth development.

These data indicate that the glycoprotein of the teeth may be considered a nutrient material for the caries-producing bacteria. With the subsequent breakdown of glucoprotein into glucose, the process of dental caries begins. From this point, the remainder of the carious process probably takes place as has been adequately postulated by the chemico-parasitic theory.

TABLE VIII

STATISTICAL INTERPRETATION OF RESULTS

DIETS	STANDARD ERROR	CURVE SLOPE VALUE-DIFFERENCE		DIFFERENCE STANDARD ERROR
Sugar-deficient	0.071	19.0	2.7	36×
Control	0.075	21.7	56.5	
Sugar-rich	0.043	75.5		753×

Since glucose acts as a medium for the cultivation of the bacterial plaque, the degree to which it is present in the teeth may be a prime inherent factor in determining caries susceptibility. However, as has been shown in this paper, the degree of caries susceptibility is modified by a series of extrinsic and intrinsic factors which vary with the particular animal in question.

SUMMARY

Three generations of Syrian hamsters were maintained on sugar-rich, sugar-deficient, and control diets for a period of 100 days and then sacrificed. Their teeth were saved and examined for dental caries and then quantitatively analyzed for the presence of glycoprotein.

The caries scores were then correlated with the glycoprotein findings, and certain relationships became apparent. Animals maintained on the low carbohydrate diet exhibited less caries than the animals maintained on the high carbohydrate diet and, also, showed a lower glycoprotein content in the teeth.

Control animals also showed less caries and less glycoprotein than the sugar-rich group, but these animals demonstrate slightly higher results in both factors when compared to the sugar-deficient group.

These results indicate that there is a positive relationship between the presence of glycoprotein in the teeth and the incidence of dental caries and that the quantity of glycoprotein present in the teeth varies with dietary factors prevailing during the time of tooth formation.

The laying down of glycoprotein apparently plays a significant role in the development of caries in the hamster. It is our hope that this will constitute data of value in stimulating further investigation of the role of diet and glycoprotein deposition in human caries.

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LACTIC ACID FORMATION BY LACTOBACILLI EXPOSED TO FLUORIDIZED ENAMEL

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MICROORGANISMS commonly encountered in dental caries secure at least part of their energy needs from the anaerobic degradation of glucose to lactic acid.[1-4] Enolase, an enzyme in the glycolytic scheme which catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate, is strongly inhibited by the fluoride ion.[5, 6] The presence of fluoride therefore inhibits glycolysis and the formation of acid by certain microorganisms. This has been suggested as a means of diminishing microbial acid production in dental caries.[7]

Fluoride salts added to cultures of various oral microorganisms in concentrations as low as 0.1 μg/ml. have been shown to inhibit acid production.[1, 7-17] Fluoride solutions in concentrations as low as 4.5 μg/ml. employed as mouth rinses have also significantly inhibited acid formation in dental plaques in situ.[18-20] Prolonged use of a rinse containing 5 μg/ml. fluoride reportedly decreased the number of lactobacilli in the saliva[21] although the use of a dentifrice containing 2260 μg/ml.† of sodium fluoride produced no consistent reduction in the number of acidogenic organisms.[22]

Of greater interest is the observation that fluoridized dental enamel inhibited the formation of acid in broth cultures of streptococci[7, 23] and of mixed oral microorganisms.[9] Unfortunately, however, this finding has not been confirmed in vivo. No differences were detected between the pH of plaques on fluoridized and nonfluoridized teeth following a 10 per cent glucose rinse.[20] Individuals receiving topical fluoride applications did not exhibit a consistent reduction in the number of lactobacilli present in the saliva.[24-27]

It is possible, however, that the fluoridized enamel surface might gradually release fluoride into the immediate bacterial environment and thus induce a local diminution in lactic acid formation or a reduction in the number of viable cells. Such an inhibitory action might play a role in reducing the incidence of dental caries. The purpose of this study was therefore to attempt to evaluate the magnitude of such fluoride inhibitory action in a model system in vivo.

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†Computed from fluoride ion—not the Na F salt.

MATERIALS AND METHODS

The model acidogenic dental plaque consisted of a washed cell suspension of *Lactobacillus casei* with added glucose applied to a fluoridized and a nonfluoridized incisor in selected individuals. After 30-minute contact with the enamel surfaces, each suspension was analyzed for lactic acid to permit an evaluation of the fluoride inhibition of acid production.

The Test Organism.—Lactobacillus casei No. 4646* was selected as the test microorganism because it is associated with caries activity[18]; it possesses glycolytic enzymes[4]; it is highly acidogenic and homofermentative; and it does not oxidize lactic acid.[18] It is not assumed, however, that this organism is necessarily an etiologic agent of dental caries.

The organism was maintained on yeast extract glucose agar stab cultures.[28] The cultures were incubated at 37° C. for 24 hours and then stored in the refrigerator as stock cultures for two weeks.

The Buffer.—The buffer employed in suspending the test cells was prepared with reagent chemicals to contain:

Magnesium sulfate	0.3 mM[30, 31]
Potassium dihydrogen phosphate	4.3 mM[30, 31]
Calcium chloride	1.0 mM[30, 31]
Glacial acetic acid	200.0 mM
Potassium hydroxide	ca. 75.0 mM

Magnesium and orthophosphate are normal constituents of saliva. They were included in the buffer because the addition of magnesium and of orthophosphate has been shown to increase the fluoride inhibition of enolase for a given fluoride concentration.[29] The phosphate also provided an ionic product of calcium and phosphate normally found in saliva. The 0.2 M. acetate buffer was compatible with cellular activity of the microorganisms and provided a buffer capacity approximating that of the natural plaques in the same pH range.[18] An initial adjustment of the pH to 4.5 was selected in order to accentuate the inhibitory action of fluoride which increases with increasing hydrogen ion concentration.[6] This pH coincides with the pH minimum usually observed in dental plaques following a glucose rinse.[18, 32-34]

Cell Suspension.—A yeast extract glucose broth tube was inoculated with a wire loop from the stock culture and incubated at 37° C. for 24 hours. The tube was then centrifuged and the supernatant decanted. The cells were suspended in broth and reinoculated into 100 ml. of yeast extract glucose broth. The final broth culture was incubated at 37° C. for 20 hours, then centrifuged for 30 minutes at 500 RCF, washed and centrifuged again in an equal volume of chilled buffer. Washing the cells removed nutritive materials, thus preventing proliferation, inducing starvation and consequently increasing the sensitivity of the cells to fluoride.[6, 7]

The centrifuged cells were transferred in a small residuum of the supernatant to a Hopkin's vaccine tube and centrifuged for 30 minutes at 500 RCF.

*Faith P. Hadley, University of Michigan. Strain C 3, from dental caries. Previously listed as *Lactobacillus acidophilus*.

The supernatant and the upper grayish layer of packed cells, composed largely of nonviable or inactive microorganisms, were removed from the vaccine tube by aspiration.[18]

Chilled buffer with 1 M. glucose was dispensed into the refrigerated vaccine tube from a microburette* immediately before use. Sufficient buffer was added by volume to the packed cells to give a 25 per cent cell suspension. The cells were suspended in the buffer with a micropipette and transferred to a depression plate.

Selection of Subjects.—Evaluation of bacterial acid production on fluoridized and nonfluoridized enamel in situ offered the advantage of control on the contralateral tooth. Biologic variations encountered in random selection of extracted teeth were thus greatly reduced. This design also allowed the assay of caries experience and of previous exposure to fluorides. Finally, possible postmortem changes in the properties of enamel and dentin were avoided.

Twelve adult males, 20 to 39 years of age, with intact vital upper first incisors and with no history of previous contact or treatment with fluorides were selected as experimental subjects. The number of decayed, missing, and filled teeth (DMF teeth) was recorded as an indication of the caries experience of each subject.

Preparation of the Teeth.—The upper central incisors were isolated with rubber dam and cleaned with flour of pumice on a rotating rubber cup. A 4 per cent (w/v) solution of sodium fluoride (unbuffered, pH 7.5) was applied topically to one incisor with a cotton pellet for a period of 20 minutes. A 5.56 per cent (w/v)† solution of sodium chloride (unbuffered, pH 7.0) was applied in the same manner to the contralateral tooth as a control. The teeth were then wiped dry and finally rinsed with tap water.

At varying intervals after the application of fluoride the incisors were again isolated with rubber dam and cleaned and dried with a solution of ether-alcohol. With the subject in a supine position, a silicone‡ coated aluminum cylinder (4.25 mm. inside diameter and 4 mm. in height) was attached with sticky wax to the labial surfaces of each of the prepared teeth.

Incubation of the Suspensions.—The cell suspension was taken up in the microburette and 8 microliters of the suspension dispensed into each of the aluminum cylinders (Fig. 1). The cylinder design permitted the fluid to assume a plane surface and thus provided a rather uniform covering of the exposed enamel to the depth of the 0.5 to 0.6 mm. This depth corresponds to the approximate thickness of dental plaques.[18] No attempt was made to stir the suspensions in the cylinders since dental plaques are stagnant.[18] Each cylinder was covered with a ball bearing to minimize evaporation. The suspensions were left in place for 30 minutes.

At the termination of the experiment, 0.05 ml. of 10 per cent copper sulfate solution was added to each cylinder from the microburette. The inactivated,

*Syringe Microburet Model SB-1, Micrometric Instrument Co., 2891 East 79th St., Cleveland 4, Ohio. Delivers 0.002 ml. with 1 per cent accuracy as calibrated by the India ink dilution method.

†Provides the same anion concentration as the fluoride solution.

‡Silicone insulating compound (Dow-Corning 4 Compound).

diluted cell suspensions were then taken up in syringe-controlled micropipettes and aliquots dispensed for the determination of lactic acid.

Determination of Lactic Acid.—The colorimetric method of Barker and Summerson[35] with modifications in the removal of sugar and color development by Markus[36] was employed in the determination of lactic acid.

Determinations were made simultaneously on duplicates of 2 standards (2 μg and 8 μg) and 2 unknowns. Requisite quantities of the standard solution and 0.01 ml. aliquots of the unknowns were delivered into separate, scrupulously clean, 10 by 75 mm. test tubes.

Twenty-four hundredths (0.24) milliliter of 10 per cent copper sulfate was added to each tube and the mixture neutralized with 0.24 ml. of 3 per cent sodium hydroxide. Four-tenths milliliter (0.4) of 10 per cent lime suspension and sufficient distilled water to make 2.0 ml. final volume were added to each tube. The tubes were thoroughly shaken. The removal of glucose was considered complete in 4 hours.

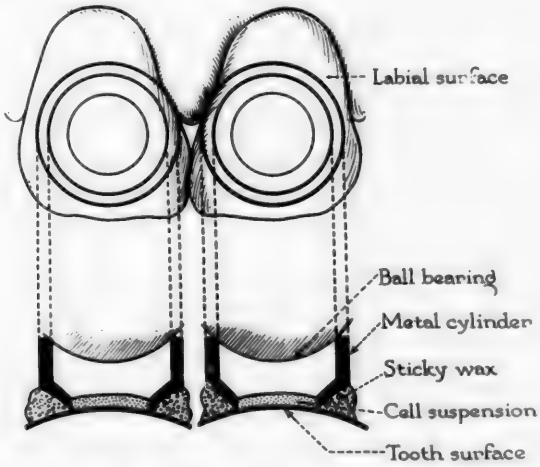

Fig. 1.—Design and arrangement of metal cylinders for incubating microsuspensions of bacterial cells on enamel surfaces in situ.

The samples were centrifuged for several minutes to remove suspended particles. One milliliter of the supernatant was carefully pipetted into a scrupulously clean, 18 by 150 mm. Pyrex test tube containing a clean, dry glass bead. Six milliliters of concentrated sulfuric acid containing 0.033 per cent (w/v) copper sulfate were rapidly delivered into each tube from a 10-milliliter syringe. The tubes were shaken vigorously and allowed to stand for exactly 5 minutes. After 5 additional minutes of cooling in tap water, 0.05 ml. of 1 per cent p-hydroxydiphenyl solution was delivered into each tube from the microburette without touching the walls. The tubes were shaken vigorously and allowed to stand for 4 hours. The optical density of the solutions was read in a Coleman Junior spectrophotometer at a wave length of 565 mμ. All manipulations on each sample were performed at precisely controlled intervals. Because of the strict adherence of the analytical system to Beer's

law, the values for optical density could be converted into micrograms of lactic acid by computation from the standards (Fig. 2). Good recovery of lactic acid from body fluids has been reported by this method.[35, 36]

The mean acid production in a series of 5 cell suspensions simultaneously prepared and dispensed as described was 1.57×10^{-9} M/μl./minute with the standard deviation $\pm 0.11 \times 10^{-9}$ M/μl./minute.

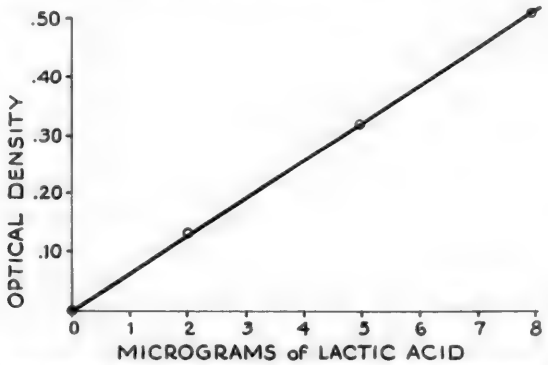

Fig. 2.—Linear relationship between color intensity and concentration of lactic acid as obtained with the Coleman Junior spectrophotometer.

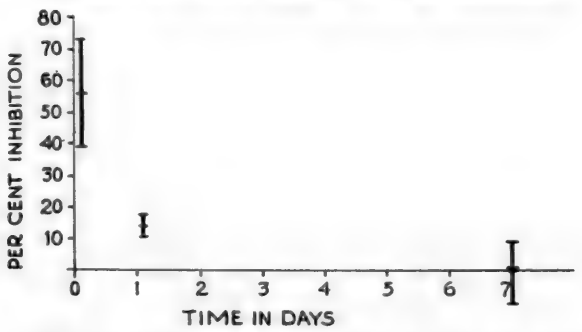

Fig. 3.—Duration of fluoride inhibition of lactic acid formation.

FINDINGS

There was a significant difference in the lactic acid formed by cell suspensions exposed to fluoridized and nonfluoridized enamel immediately after topical treatment—even though the degree of difference varied considerably in different subjects.

The suspensions which had been in contact with the water-rinsed, treated teeth contained an average of 56 per cent less lactic acid than the suspensions on the contralateral control teeth. This difference was statistically significant at the 5 per cent ($p < 0.05$) level by the analysis of variance (Table I).

The differences in acid formation *between subjects* were significant beyond the 0.1 per cent level ($p < 0.001$). They probably reflect variations in bacterial cultures and ambient temperatures and do not interfere with the results of the experimental procedure.

TABLE I

FORMATION OF LACTIC ACID BY SUSPENSIONS OF *L. casei* ON FLUORIDIZED AND NONFLUORIDIZED ENAMEL IMMEDIATELY AFTER TREATMENT
(10^{-9} M. of lactic acid/μl./min.)

NO.	SUBJECT	AGE	DMF TEETH	TEETH NF*	TEETH F†	DIFFERENCE	PER CENT INHIBITION
1	A. B.	31	18	2.60	0.68	-1.88	73.7
				2.50	0.66		
2	P. B.	28	14	2.25	1.46	-0.76	33.3
				2.29	1.57		
3	E. V.	39	7	2.85	1.28	-1.46	53.0
				2.64	1.30		
4	W. S.	22	9	3.75	1.33	-2.25	60.6
				3.65	1.58		
	Means			2.82	1.23	-1.58	(56.2)

Analysis of Variance of the Data

SOURCE OF VARIANCE	DF‡	SUM OF SQUARES	MEAN SQUARES	VARIANCE RATIO (F)	
Between groups	1	10.0330	10.0330	$\frac{10.0330}{0.4095}$	= 24.5 ($p < 0.05$)
Between subjects	3	1.9803	0.6601	$\frac{0.6601}{0.0088}$	= 74.8 ($p < 0.001$)
Interaction (groups × subjects)	3	1.2285	0.4095	$\frac{0.4095}{0.0088}$	= 46.6 ($p < 0.001$)
Between duplicates	8	0.0706	0.0088		
Total	15	13.3124			

*NF: Nonfluoridized.
†F : Fluoridized.
‡DF: Degrees of freedom.

The percentage differences in acid formation between fluoridized and nonfluoridized teeth also varied from experiment to experiment. This variation was significant beyond the 0.1 per cent level ($p < 0.001$). Since there was only a slight apparent correlation between age and inhibition of acid formation, it is clear that some factor—as yet unknown—affected the experimental comparisons.

The inhibition of lactic acid formation observed in cell suspensions immediately after fluoridization of the enamel showed a rapid decline as the interval between application and analysis was increased (Fig. 3).

Thus, cell suspensions in contact with the teeth 24 hours after treatment formed on the average only 14 per cent less lactic acid than the suspensions on control teeth. This difference was statistically significant at the 5 per cent ($p < 0.05$) level by the analysis of variance (Table II). At 7 days no consistent difference in lactic acid formation in suspensions on fluoridized and nonfluoridized teeth was detected (Table III).

Significant differences between subjects were observed in both the series at 24 hours and that at 7 days. Again these differences do not interfere with the results of the experimental procedure.

The failure of the percentage differences between acid formation on fluoridized and nonfluoridized enamel to be identical from experiment to experiment was not significant in the 24-hour series and scarcely significant at the 5 per cent level ($p < 0.05$) in the 7-day series.

TABLE II

FORMATION OF LACTIC ACID BY SUSPENSIONS OF *L. casei* ON FLUORIDIZED AND NON-FLUORIDIZED ENAMEL TWENTY-FOUR HOURS AFTER TREATMENT
(10^{-9} M of lactic acid/μl./min.)

NO.	SUBJECT	AGE	DMF TEETH	TEETH NF	TEETH F	DIFFERENCE	PER CENT INHIBITION
1	J. M.	25	12	3.28	2.85	-0.42	12.5
				3.44	3.03		
2	J. A.	21	17	3.36	2.77	-0.59	17.1
				3.45	2.87		
3	J. S.	24	16	3.03	2.66	-0.51	16.0
				3.31	2.66		
4	H. G.	21	14	2.70	2.40	-0.24	8.9
				2.65	2.47		
	Means			3.15	2.71	-0.44	(14.0)

Analysis of Variance of the Data

SOURCE OF VARIANCE	DF	SUM OF SQUARES	MEAN SQUARES	VARIANCE RATIO (F)	
Between groups	1	0.7700	0.7700	$\frac{0.7700}{0.0254}$	$= 30.2$ ($p < 0.05$)
Between subjects	3	0.8901	0.2967	$\frac{0.2967}{0.0089}$	$= 33.4$ ($p < 0.001$)
Interaction (groups × subjects)	3	0.0763	0.0254	$\frac{0.0254}{0.0089}$	$= 2.9$ ($p < 0.2$)
Between duplicates	8	0.0710	0.0089		
Total	15	1.8074			

TABLE III

FORMATION OF LACTIC ACID BY SUSPENSIONS OF *L. casei* ON FLUORIDIZED AND NON-FLUORIDIZED ENAMEL SEVEN DAYS AFTER TREATMENT
(10^{-9} M of lactic acid/μl./min.)

NO.	SUBJECT	AGE	DMF TEETH	TEETH NF	TEETH F	DIFFERENCE	PER CENT INHIBITION
1	M. B.	35	16	1.56	1.63	+0.15	
				1.63	1.85		
2	L. L.	34	14	1.69	(1.65)*	-0.04	2.36
				1.69	1.65		
3	M. Z.	21	3	2.82	2.63	-0.34	12.30
				2.73	2.24		
4	S. K.	22	19	2.54	2.70	+0.19	
				2.48	(2.70)*		
	Means			2.14	2.13	-0.01	(0.47)

Analysis of Variance of the Data

SOURCE OF VARIANCE	DF	SUM OF SQUARES	MEAN SQUARES	VARIANCE RATIO (F)	
Between groups	1	0.0005	0.0005		
Between subjects	3	3.5063	1.1688	$\frac{1.1688}{0.0136}$	$= 86.0$ ($p < 0.001$)
Interaction (groups × subjects)	3	0.1738	0.0579	$\frac{0.0579}{0.0136}$	$= 4.3$ ($p < 0.05$)
Between duplicates	8	0.1086	0.0136		
Total	15	3.7892			

*Data supplied.

DISCUSSION

A direct measure of the amount of fluoride released from the enamel into the test cell suspensions was not attempted. The inhibition caused by the addition of known concentrations of fluoride to test suspensions in vitro, however, permitted an estimate of the fluoride presumably released by the enamel under in situ test conditions.

It was found that fluoride added to 8 microliters of cell suspension to give a final concentration of 10 μg/ml. inhibited the formation of lactic acid 66 per cent. Two hundred and fifty μg/ml. of fluoride essentially prevented the formation of acid.

It may be inferred that the inhibitory effects observed in situ were the result of fluoride concentrations somewhat less than 10 μg/ml.

TABLE IV

INHIBITION OF LACTIC ACID PRODUCTION WITH INCREASING CONCENTRATIONS OF FLUORIDE

CONCENTRATION OF FLUORIDE (μg/ml.)	LACTIC ACID FORMED (10^{-9} M/μl./MIN.)	PER CENT INHIBITION
0	1.81	0.0
	1.83	
10	0.62	65.7
	0.63	
250	0.07	94.1
	0.14	

The rapid disappearance of the fluoride inhibition of lactic acid production observed in these tests probably excludes this mode of fluoride action as a significant factor in the reduction of dental caries incidence following topical fluoride treatments. Protection against the initiation of dental caries persisting one year and perhaps longer following the clinical application of fluorides to the teeth[37] is hardly related to an inhibitory effect demonstrable for only a matter of hours.

Nevertheless, the magnitude of the inhibitory action over a short interval may be highly significant for the protective action of fluorides in potable water supplies. It may well be that fluorides present in the water in high dilution (1 μg/ml.) are concentrated on the enamel surface by adsorption and are released under the hydrogen ion conditions of caries attack thereby limiting its destructive potential. Continuous exposure to fluoridated water thus might confer protection against caries after the formation and eruption of the teeth. Such a possibility certainly deserves experimental evaluation.

The marked diminution with time in the degree of inhibition of lactic acid production may be attributed to either a partial loss of the fluoride initially retained on the enamel or to an increasing unavailability of the retained fluoride. It emphasizes the critical importance of testing fluoride applications in situ—after equilibration of the treated surfaces with the oral fluids. This may well apply to the evaluation of other dental therapeutic agents.

The experimental design used in the present study permits the accumulation of exact data under closely approximated clinical conditions, and thus bridges the gap between in vitro testing and epidemiologic surveys on large populations.

The experimental methods employed in this study should prove useful in a further investigation of the clinical efficacy of different fluoride salts, other caries control agents, and for additional studies into the mechanism of the caries process.

SUMMARY AND CONCLUSIONS

1. Topical fluoride retained on vital intact enamel inhibits lactic acid formation in microsuspensions of glycolytically active, washed cells of *L. casei* in an acetate buffer.

2. The observed inhibition of lactic acid formation requires a fluoride ion concentration on the enamel surface of less than 10 μg/ml.

3. The degree of inhibition of lactic acid production decreases sharply in 24 hours of exposure of the teeth to saliva and completely disappears within 7 days.

It may be concluded that the clinical efficacy of topical fluorides in reducing the incidence of dental caries is probably not associated with an inhibition of glycolytic enzyme activity.

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THE STATE OF FLUORIDE IN DRINKING WATER

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THE widespread use of sodium silicofluoride in fluoridating drinking water has made it important to determine the state of the fluoride in such water, specifically, how much is fluoride ion, how much, if any, is unchanged silicofluoride, how much is fluoride bound to other ions. If all or nearly all of the fluoride is the ion, F^-, the great body of information about the biologic effects of fluorides[1, 2] can be brought forward as a guarantee of safety. If considerable amounts of silicofluoride remain, a question can legitimately be raised since comparatively little work has been done on the biologic effects of silicofluorides. This paper presents some experimental results permitting the conclusion that no silicofluoride as such exists in the drinking water. The calculations and interpretations, while physicochemical, are described in sufficient detail to be intelligible to those without formal training in physical chemistry.

When sodium silicofluoride is added to water, there is an instantaneous and complete dissociation into sodium and silicofluoride ions. $Na_2SiF_6 \rightarrow 2Na^+ + SiF_6^=$. Thereafter,[3, 4] the silicofluoride ions dissociate slowly to un-ionized, dissolved silicon tetrafluoride and fluoride ions, according to the equilibrium:

(1) $SiF_6^= \rightleftarrows SiF_4 + 2F^-$.

The silicon tetrafluoride promptly hydrolyses to give silicon dioxide, probably hydrated as silicic acid, H_2SiO_3, and fluoride and hydrogen ions.

(2) $SiF_4 + 3H_2O \rightleftarrows H_2SiO_3 + 4H^+ + 4F^-$.

The latter ions may combine to some extent to give un-ionized hydrogen fluoride.

(3) $H^+ + F^- \rightleftarrows HF$.

It is obvious, then, that after silicofluoride is introduced into water some fluoride may be in any or all of the following states: $SiF_6^=$, SiF_4, F^-, and HF.

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The amount of fluoride in each of these states depends on the equilibrium constants* of reactions (1), (2), and (3), which are, respectively,

$$K_1 = \frac{(SiF_4)(F^-)^2}{(SiF_6^=)}$$

$$K_2 = \frac{(H_2SiO_3)(H^+)^4(F^-)^4}{(SiF_4)}$$

$$K_{HF} = \frac{(H^+)(F^-)}{(HF)}$$

The hydrolysis constant for the over-all reaction is given by combining (1) and (2).

$$SiF_6^= + 3HOH \rightleftarrows H_2SiO_3 + 4H^+ + 6F^-$$

$$K_h = \frac{(H_2SiO_3)(H^+)^4(F^-)^6}{(SiF_6^=)}$$

$$= \frac{(F^-)^2(SiF_4)}{(SiF_6^=)} \times \frac{(H_2SiO_3)(H^+)^4(F^-)^4}{(SiF_4)}$$

$$= K_1K_2$$

In addition, some fluoride might be combined with metallic ions as metallo-fluoride complexes; e.g., CaF^+, MgF^+, FeF^{++}, AlF^{++}. These reactions may be described in general as follows: $M^{+m} + zF^- \rightleftarrows MF_z^{m-z}$. The amount of a given metallo-fluoride complex, MF_z, present depends upon its stability constant,

$$K_{MF_z} = \frac{(MF_z^{m-z})}{(M^{+m})(F^-)^z}$$

as well as upon K_1, K_2, and K_{HF}.

The fraction of the total fluoride in each of the possible forms in a given water sample may be estimated from a knowledge of these equilibrium constants; viz., K_1, K_2, K_{HF}, and K_{MF_z}.

HYDROLYSIS OF SODIUM SILICOFLUORIDE

The equilibrium constant for reaction (1), the initial slow dissociation of the silicofluoride ion, viz., $K_1 = \frac{(SiF_4)(F^-)^2}{(SiF_6^=)}$ was estimated by Rees and Hudleston[4] to be $1.1 \pm 0.2 \times 10^{-6}$ at 20° C. and by Ryss[5] to be 0.65×10^{-6} at 11° C.

The constant, $K_2 = \frac{(H^-)^4(F^-)^4(H_2SiO_3)}{(SiF_4)}$, for reaction (2), the hydrolysis of SiF_4, was determined by us experimentally. Because one of the reaction

*The parentheses in the equilibrium constant expressions indicate that the chemical species are expressed in terms of activities, or "effective concentrations," rather than in terms of actual concentrations. The activity of a species is equal to the actual concentration times a correction factor called the activity coefficient. The activity coefficient of a given species owes its existence to the decrease in the mobility of that species when other electrically charged, or polar, particles exert electrical attractive forces. These forces increase with an increase in the total concentration of charged particles in the solution.

At infinite dilution the charged particles are so far apart that they exert no electrical effects on each other, the activity coefficient of each particle is unity, and the activities equal the concentrations. As the solution becomes more concentrated, with respect to charged particles, the mobility of each decreases, their activity coefficients become less than unity, and their activities become less than their actual concentrations.

An equilibrium constant expressed in terms of activities is independent of the concentrations of charged particles because of the inclusion of the electrical effects in the activity coefficients.

products is the hydrogen ion, in the absence of a potent buffer the extent of this reaction can be conveniently measured by taking the pH of the solution some time after the silicofluoride is added. In fact, however, a number of reactions occur that tend to buffer the solution, but the concentration of each constituent of the solution can be expressed in terms of the hydrogen ion concentration. This phenomenon was utilized experimentally.

The pH values of serial dilutions of a 0.0109 molar sodium silicofluoride solution were measured after the solutions had been shaken for 24 hours at 34° C. and then had been allowed to stand at room temperature, 29° C. for an additional 24 hours, care being taken to exclude CO_2 at all times.

Fluoride can theoretically be found in these solutions in one or more of the following states: $SiF_6^=$, SiF_4, F^-, and HF. The problem is to find the relative amounts of these forms with special reference to 2 points: (a) does any significant concentration of $SiF_6^=$ or SiF_4 exist, and (b) is essentially all of the fluoride present as F^-?

Let us introduce the symbols C_0, x and y such that:

C_0 = initial stoichiometric molarity of Na_2SiF_6.
x = gram ions of $SiF_6^=$ dissociated by reaction (1).
y = molarity of H_2SiO_3 produced by step (2).

We may then represent the equilibrium concentrations of the fluoride-containing constituents in a solution of sodium silicofluoride as follows:

$$[SiF_6^=] = C_0 - x$$
$$[SiF_4] = x - y$$
$$[F^-] = 2x + [H^+]$$
$$[HF] = 4y - [H^+]$$

where the brackets indicate concentrations in moles (or gram ions) per liter.

If we let f_{H^+}, f_{F^-}, and f_{HF} represent the activity coefficients of hydrogen ion, fluoride ion, and hydrogen fluoride, respectively, we may express the thermodynamic dissociation constant of hydrogen fluoride as follows:

$$K_{HF} = \frac{f_{H^+}[H^+] \cdot f_{F^-}[F^-]}{f_{HF}[HF]}$$

Since the hydrogen fluoride molecule is uncharged we may set f_{HF} equal to unity in dilute solutions. Then

$$(4) \quad K_{HF} = \frac{f_{H^+}[H^+] \cdot f_{F^-}\{2x + [H^+]\}}{4y - [H^+]}.$$

Similarly, the equilibrium constant, K_1, may be expressed as:

$$K_1 = \frac{f_{SiF_4}[SiF_4] \cdot f_F^2[F^-]^2}{f_{SiF_6^=}[SiF_6^=]}.$$

Letting f_{SiF_4} equal unity,

$$(5) \quad K_1 = \frac{\{x - y\} \cdot f_{F^-}\{2x + [H^+]\}^2}{4y - [H^+]}.$$

At low ionic strength, the Debye-Huckel equation[6] for the activity coefficient of an individual ion justifies the following approximations: $f_1 = f_{H^+} = f_{F^-} = f_{SiF_6^{=}}^{1/4}$, where f_1 is the activity coefficient of a monovalent ion. If these approximations are made in equations (4) and (5) and the two equations are then solved simultaneously, equation (6) results.

$$(6)\quad \{8f_1^4(H^+) - 16f_1^3K_{HF}\}x^3 + \{12f_1^3(H^+)^2 - 12f_1K_{HF}(H^+)\}x^2 + \{6f_1^2(H^+)^3 - 4K_{HF}K_1f_1^5\}x + f_1(H^+)^4 + (H^+)^3K_{HF} + 4K_{HF}K_1f_1^5C_0 = 0.$$

This equation, cubic in x, may be solved for x at any given value of C_0 if K_1, K_{HF}, f_1, and (H^+) are known. The K_1 used in these calculations was 1×10^{-6}, i.e., the value given by Rees and Hudleston[4] for 20° C. No attempt was made to correct this value to 29° C. because it is believed that the values reported for 11° and 20° by Ryss[5] and Hudleston, respectively, indicate that the change in K_1 from 20° to 29° approaches the experimental error in Hudleston's value for 20° C.

The value 6.2×10^{-4} for K_{HF} at 29° C., was determined by interpolation of the data of Broane and DeVries.[7]

The hydrogen ion activity was obtained by applying the equation $pH = -\log_{10}(H^+)$.

The activity coefficient, f_1, was estimated from the Debye-Huckel[6] equation, $\log f_1 = \dfrac{-0.51\mu^{1/2}}{1 + 0.33A_0\mu^{1/2}}$ where μ is the ionic strength and A_0 is a parameter.* A value of 6 was used for A_0 in our calculations. It can easily be shown that this approximation involves an error of no greater than 5 per cent in the assumption that $f_1 = f_{F^-} = f_{H^+}$ at the highest ionic strength used. The ionic strengths used in these calculations were based on complete ionization, without subsequent hydrolysis, of the original sodium silicofluoride to sodium and silicofluoride ions; i.e., $\mu \cong 3C_0$. The maximum error in f_1 as a result of this assumption is less than 5 per cent even at the highest ionic strength used. This produces about 25 per cent uncertainty in the calculated K_2.

The greatest error in the determination of K_2, however, is probably in the pH measurements and in the assumption that $pH = -\log_{10}(H^+)$. Considering that the fourth power of (H^+) is involved and (H^+) is also part of the calculated (F^-), which occurs to the sixth power in the over-all hydrolysis constant, $K_h = K_1K_2$, it appears obvious that no better than a factor of 2 accuracy can be claimed for the calculated values of K_2 and K_h. For example, an error of 10 per cent in the hydrogen ion calculation would confer a 40 per cent error in the K_2 value and a still greater error in the K_h value.

*This equation is derived by relating the activity coefficient of an ion to its electrical potential. This electrical potential depends on a term, μ, called the ionic strength, which is equal to $\sum \frac{C_iZ_i^2}{2}$, where i is the number of different ionic species in solution, C_i is molar concentration of species i, and z is valence of species i. The empirically determined constant A_0 has the dimension of length in angstroms and may be regarded as related to the hydrated-ion radius. The A_0 values for hydrogen and fluoride ions are 9 and 3.5, respectively.[8] With the exception of the value for hydrogen ion, A_0 rarely exceeds 6 for univalent ions.[8]

The results, averages of duplicate determinations, are presented in Table I. Note that as the initial silicofluoride concentrations decreased one hundredfold in the five solutions studied, the pH remained relatively constant. The average value attained for K_2 was $7(\pm 3) \times 10^{-26}$, which when multiplied by $K_1 = 10^{-6}$ gives a value of $7(\pm 3) \times 10^{-32}$ for the over-all hydrolysis constant, K_h. Values of the order of 10^{-27} have been reported for the hydrolysis constant of the silicofluoride ion by Kubelka and Pristoupil[9] and by Ryss and Bakina[10] but they defined the hydrolysis constant as $\frac{(H^+)^4(F^-)^6}{(SiF_6^=)}$ because their solutions were saturated with respect to silicic acid. We do not believe the H_2SiO_3 activity can be ignored since the solubility of H_2SiO_3 probably changes with the silicofluoride concentration; i.e., a constant not containing the H_2SiO_3 activity would give no information concerning a dilute sodium silicofluoride solution which is not saturated with respect to any silica-containing substance.

TABLE I

HYDROLYSIS CONSTANT OF SILICOFLUORIDE ION

(ALL CONCENTRATIONS ARE EXPRESSED IN MOLES PER LITER)

$C_s \times 10^3$	pH	μ	f_1	$X \times 10^3$	$[H_2SiO_3] \times 10^3$	$[SiF_4] \times 10^3$	$[SiF_6^=] \times 10^3$	$[F^-] \times 10^3$	$K_2 \times 10^{26}$ and $K_h \times 10^{32}$
10.94	3.60	.033	.86	1.24	.31	.93	9.7	2.8	4
5.390	3.50	.016	.89	0.99	.36	.63	4.4	2.0	11
1.094	3.50	.0033	.94	0.52	.25	.27	.57	1.4	3
.539	3.43	.0016	.96	0.37	.26	.11	.43	1.2	9
.109	3.61	.0003	.98	.108	.108	$< 2 \times 10^{-4}$	$< 1 \times 10^{-3}$	Average	7 ± 3

Drinking water invariably has a pH which is at least 5 and usually is between pH 6 and 8. The usual practice in artificial fluoridation is to maintain the total fluoride in the water at about 1 ppm or 5×10^{-5} gram atoms per liter. Many water supplies in western states have or have had 5 to 8 ppm total fluoride and in a very few cases concentrations as high as 16 ppm total fluoride have been found. These values will be used to illustrate possible variations in the calculations that follow.

The extent of hydrolysis of sodium silicofluoride in a given water supply depends on the fluoride ion content and on the pH (equations 1 and 2).

Rearrangement of the K_2 and K_h expressions gives the following ratios:

$$(7) \quad \frac{(H_2SiO_3)}{(SiF_4)} = \frac{K_2}{(H^+)^4(F^-)^4}.$$

$$(8) \quad \frac{(H_2SiO_3)}{(SiF_6^=)} = \frac{K_h}{(H^+)^4(F^-)^6}.$$

The $(H_2SiO_3)/(SiF_4)$ ratio and the $(H_2SiO_3)/(SiF_6^=)$ ratio of water having a pH of 5 and fluoride ion content of 16 ppm are calculated from equations (7) and (8) to be 2×10^7 and 3×10^7, respectively. These very large numbers (2 or 3×10^7) indicate that only negligible amounts ($< 10^{-10}$ moles per liter) of SiF_4 or of $SiF_6^=$ can be present under these conditions and that essentially all of the fluoride is F^-, HF, or metal-complexed F.

These ratios would be even higher for the smaller fluoride content in artificially fluoridated water (i.e., 1 ppm) or at the higher pH (i.e., 6 to 8) usually found for surface waters. As an example, the ratios for the Rochester, New York, water supply are calculated to be 10^{+20} and 10^{+25}, respectively, assuming the fluoride content to be 1 ppm and pH to be 7.5.[11] There is no SiF_4 or $SiF_6^=$ present in Rochester water, specifically, less than 1 molecule of either per liter of water. There is no question of toxicity of SiF_4 or $SiF_6^=$ under such conditions.

The ratio $(F^-)/(HF)$ in a fluoride-containing solution is $K_{HF}/(H^+)$. The constant K_{HF} is 7.9×10^{-4} at 15° C. and 5.6×10^{-4} at 35° C. Hence, at pH 5, the $(F^-)/(HF)$ ratio is 79 at 15° C. and 56 at 35° C. The hydrogen fluoride content of fluoridated water in the temperature range 15° to 35° C. is, then, only about 1.5 per cent of the fluoride ion content at pH 5 and decreases about tenfold per unit increase in pH (see the text table).

pH	(HF)/(F^-) RATIO	% HF OF TOTAL FLUORIDE
5	0.015	1.5
6	0.0015	0.15
7	0.00015	0.015
8	0.000015	0.0015

It appears a certainty therefore that, in any water supply with pH 5 or higher and having a total fluoride content of 16 ppm or less, all silicofluoride is completely hydrolysed to silicic acid, fluoride ion, and hydrogen fluoride and that the latter compound constitutes only a very small fraction of the total fluoride.

METALLO-FLUORIDE COMPLEXES IN WATER SUPPLY*

Metallic ions found in every water supply tend to complex fluoride; the greater their concentrations and their stability constants, the more fluoride is bound.

The known stability constants, defined as $K_{MF} = \frac{(MF)}{(M)(F^-)}$, for complexes containing one fluoride ion at 25° C. are quoted from Paul[12] in Table II, column 2. The larger the value of K_{MF} the greater the tendency to form a complex. Paul found that a linear relation exists for log K vs. {(metal ion valence)^2/metal ion radius} for all the known metallo-monofluoride complex stability constants except those of thorium (ThF^{+3}), scandium (ScF^{+2}), and tin (SnF^+). We have estimated the constants for the monofluoride complexes of Li^+, Mn^{+2}, Ni^{+2}, Sr^{+2}, Ti^{+3}, and Sn^{+4} by assuming that they would obey the linear relation found by Paul. The ionic radii used were taken from those tabulated by Lipscomb.[13]

To gain some appreciation of the possible importance of the metallo-fluoride complexes, the concentrations of these metallic ions that would bind 1 per cent of the fluoride in the water can be calculated and compared with the concentrations found in certain water supplies. The activity of free metal ions,

*In all the following calculations the equilibrium constants are for 25°C. Hence, all discussions refer to 25°C.

(M), in a solution containing a given $(MF)/(F^-)$ ratio may be obtained by rearrangement of the stability constant expression to $(M) = \frac{(MF)}{(F^-)K_{MF}}$. The (M) values thus calculated for a $(MF)/(F^-)$ ratio of 0.01 are presented in Table II, column 3; these values give the lowest concentrations (activities) of each ion that would complex 1 per cent of the total F^-. The same concentrations expressed in ppm are given in column 4. Lower concentrations can be neglected on the basis that complex ions accounting for less than 1 per cent of the total fluoride can hardly have biologic significance.

TABLE II

ION^{+m}	K_{MF}	FREE M CONC. CALCULATED TO COMPLEX 1% OF TOTAL F		MAXIMUM M CONC. FOUND BY BRAIDECH AND EMERY	$K_{sp}/(OH^-)^m$† AT pH 5
		GRAM IONS/LITER	ppm	ppm	GRAM IONS/LITER
*Li^+	1	1×10^{-2}	70	0.2	
Ag^+	2.3	4.4×10^{-3}	480	0.1	
Ba^{+2}	< 2.8	$< 3.5 \times 10^{-3}$	< 480	0.4	
Hg_2^{+2}	< 3.4	$< 3.0 \times 10^{-3}$	< 1200		
Pb^{+2}	< 5.4	$< 1.8 \times 10^{-3}$	< 373	0.18	
*Sn^{+2}	10	1×10^{-3}	88	10	
Ca^{+2}	< 11	$< 9 \times 10^{-4}$	< 36	82	10^{+12}
Cu^{+2}	17	6.0×10^{-4}	38	0.6	
Zn^{+2}	18	5.6×10^{-4}	36	0.2	
*Mn^{+2}	30	3.3×10^{-4}	18	0.3	
Hg^{+2}	36	2.8×10^{-4}	56		
Mg^{+2}	66	1.5×10^{-4}	3.6	39	10^{+6}
*Ni^{+2}	1×10^2	1×10^{-4}	6	0.3	
La^{+3}	3.6×10^3	2.8×10^{-6}	.39		
Ce^{+3}	9.8×10^3	1.0×10^{-6}	.14		
Gd^{+3}	1.8×10^4	5.6×10^{-7}	.088		
In^{+3}	4.3×10^4	2.3×10^{-7}	.026		
*Sn^{+2}	7×10^4	1.4×10^{-7}	.017	0.1	3×10^{-9}
Cr^{+3}	1.6×10^5	6.2×10^{-8}	.0032	0.04	6.7×10^{-4}
Ga^{+3}	7.3×10^5	1.4×10^{-8}	9.8×10^{-4}		
Be^{+2}	7.7×10^5	1.3×10^{-8}	1.1×10^{-4}		
Ti^{+3}	10^6	1×10^{-8}	5×10^{-4}	0.07	10^{-13}
Fe^{+3}	1.1×10^6	9.1×10^{-9}	5×10^{-4}	1	6×10^{-11}
Al^{+3}	1.0×10^7	1.0×10^{-9}	2.7×10^{-5}	1	5×10^{-6}
Sc^{+3}	1.2×10^7	8.3×10^{-10}	3.7×10^{-5}		
Pu^{+4}	8.7×10^7	1.2×10^{-10}	2.9×10^{-5}		
Th^{+4}	4.5×10^8	2.2×10^{-11}	5.1×10^{-6}		
Zr^{+4}	6.3×10^9	1.6×10^{-11}	1.4×10^{-6}		
*Sn^{+4}	3×10^{11}	3×10^{-14}	3×10^{-12}	0.1	10^{-21}

*Estimated (see text).

†Maximal free metal ion concentration (at pH 5) on the basis of precipitation as hydroxide.

Braidech and Emery[14] analyzed spectrographically monthly composites of daily samples for February and March of 1934 from municipal water-purification plants of 24 cities located throughout the United States and considered to be typical of the variety of water supplies in this country. The maximal concentration found for each metal ion is given in Table II, column 5. They believed that other metals than those reported occurred only in trace amounts.

A comparison of the data in columns 4 and 5 shows that of those metals listed the only ones possibly present in sufficient concentration to complex at least 1 per cent of the fluoride are calcium, magnesium, tin, chromium, aluminum, iron, and titanium. In the discussion that follows it will be shown that, of these, only calcium and magnesium actually complex more than 1 per cent of the F^-.

It is not the total metal content of the water but the *free* metal ion content that determines the amount of fluoride complexed. Metallic ions tend to hydrolyze; the weaker the hydroxide, the greater the hydrolysis and the lower the *free* metal ion concentration. The maximal *free* metal ion content in a water sample at any given pH can be calculated from the solubility product of the hydroxide by the equation: $(M^{+m}) = Ksp/(OH^-)^m$. In column 6, Table II, are the $Ksp/(OH^-)^m$ ratios at pH 5 for the indicated metals. The K_{sp} values were taken from Latimer.[15] At higher pH, these ratios would be even smaller. It is evident from a comparison of columns 3 and 6 that the concentrations of Sn^{+2}, Sn^{+4}, Ti^{+3}, and Fe^{+3} ions which can exist at pH 5 or above is much smaller than the concentrations which would exist when $(MF)/(F^-) = 0.01$, i.e., required to complex 1 per cent of the total F. In other words, tin, iron, and titanium can be ruled out as important F complexers in drinking water because the *free* metal ion concentration will never be appreciable.

Chromium ion (Cr^{+3})—comparing columns 3 and 6—appears to have the possibility of complexing more than 1 per cent of the F^-. In columns 3 and 4 of Table II it is seen that if the chromic ion activity is 6.2×10^{-8} gram ions per liter (0.0032 ppm), the $(MF)/(F^-)$ ratio will be 0.01. The total chromium content in each water sample analyzed by Braidech and Emery was $\leqq 0.01$ ppm, except in the Indianapolis sample which contained 0.04 ppm chromium. From the stability constant of CrF_3^{+2}, one calculates that if all the chromium were in the form of Cr^{+3}, the $(MF)/(F^-)$ ratio in Indianapolis water would be about 0.13 (13 per cent) and in the other localities this ratio would be $\leqq$ 0.03 (3 per cent). However, from the equilibrium constant, 1.5×10^{-4}, for the reaction, $Cr^{+3} + H_2O = CrOH^{+2} + H^+$, it is calculable that most of the ionic chromium would be in the hydrolysed form at pH 5 and above.

Thus, $$1.5 \times 10^{-4} = \frac{(CrOH^{+2})(H^+)}{(Cr^{+3})}$$

at pH 5; $$\frac{(Cr^{+3})}{(CrOH^{+2})} = \frac{10^{-5}}{1.5 \times 10^{-4}} = \frac{1}{15}.$$

That is, only $\frac{1}{16}$ of the ionic chromium is chromic ion at pH 5. Similarly at pH 6, 7, and 8, it can be calculated that the $(Cr^{+3})/(CrOH^{+2})$ ratios are 1:150, 1:1,500, and 1:15,000, respectively. Hence, even in the Indianapolis water sample, less than 1 per cent of the fluoride would be complexed by chromium at any pH above 5.

Braidech and Emery found vanadium in only the St. Louis sample. The concentration reported was 0.01 ppm (not given in Table II). In aqueous solutions, positive vanadium ions exist only as VO^{+2} and $V(OH)_4^+$. Although the K_{MF} is not known for either of these ions, a consideration of the K_{MF}

values reported for all the monovalent and divalent ions except the relatively small beryllium ion leads to the safe assumption that K_{MF} for a vanadyl-fluoride complex is below 100. Thus, at least 5 ppm vanadium would be required to complex 1 per cent of the fluoride. We may conclude, therefore, that no vanadium-fluoride complex exists in drinking water.

Similar considerations lead to the dismissal of antimony, occurring in a concentration of 0.04 ppm in Salt Lake City water, as a complexer of fluoride.

The basis for ruling out the aluminum ion as a significant fluoride complexer is somewhat more complicated. From $(Al^{+3}) = Ksp/(OH)^{-3} = 5 \times 10^{-33}/(OH^-)^3$, one calculates that (Al^{+3}) equals 1×10^{-9} gram ions per liter, the value corresponding to $(AlF^{+2})/(F^-) = 0.01$, when $(OH^-) = 1.7 \times 10^{-8}$ gram ions per liter or pH $\cong$ 6.2. That is, any water having a pH greater than about 6 must contain less (Al^{+3}) than is required to complex 1 per cent of the F^-. At pH below 6.2, however, competition with hydroxyl or sulfate to form complex ions must also be considered. The monohydroxyl complex will not prevent the formation of the aluminum fluoride complex* but combined with the sulfate, as the $AlOHSO_4$ molecule, essentially all of the aluminum is unavailable to the F^-. The extent of this competition can be estimated from the appropriate stability constants.

$$\frac{(AlF^{+2})}{(AlOHSO_4)} = \frac{K_{AlF^{+2}}(F^-)}{K_{AlOHSO_4}(SO_4^=)/(H^+)} = \frac{7.4 \times 10^{-7}(F^-)}{1.25 \times 10^{-2}(SO_4^=)/(H^+)}.$$

The lowest sulfate concentration reported by Braidech and Emery was 3 ppm, or 3×10^{-5} gram ions per liter, in Houston. Using an even smaller value, 1 ppm for sulfate, and a pH of 5, gives $(AlF^{+2})/(AlOHSO_4) = 6 \times 10^{-5}(F^-)$. At higher pH, this ratio would be smaller. Since the total fluoride in the most highly fluoridated water considered is about 16 ppm, or 8×10^{-4} gram atoms per liter, it is apparent that no natural drinking water supply having a pH as high as 5 can contain an $(AlF^{+2})/(AlOHSO_4)$ ratio greater than 5×10^{-8}. Hence it is extremely unlikely that any aluminum fluoride complexes exist in fluoridated drinking water.

Similar calculations, using the solubility products given by Latimer for the sulfates, hydroxides, and carbonates of calcium and magnesium show that none of these anions will compete with fluoride ions for any available calcium or magnesium ions. Hence, it is probable that all the calcium and magnesium in drinking water is available for fluoride complexing according to the formulae:

$$\frac{(MgF^+)}{(F^-)} = K_{MgF^+} \times Mg^{++} \text{ content of water.}$$

$$\frac{CaF^+)}{(F^-)} = K_{CaF^+} \times Ca^{++} \text{ content of water.}$$

The magnesium and calcium contents of water both range from about 1 ppm (4×10^{-5} gram atoms per liter for Mg; 2×10^{-5} for Ca) in very soft water

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* $\frac{(AlF^{+2})}{(AlOH^{+2})} = \frac{(F^-)K_{AlF^{+2}}}{(OH^-)K_{AlOH^{+2}}} = \frac{7.4 \times 10^{-7}(F^-)}{1.4 \times 10^{-9}(OH^-)} = \frac{530\ (F^-)}{(OH^-)}$.

At pH 6 this ratio equals $5.3 \times 10^{10}(F^-)$; at pH 5, $5.3 \times 10^{11}(F^-)$. Hence, in any naturally or artificially fluoridated water which surely contains an (F^-) much greater than 10^{-3}M, the concentration of $AlOH^{+2}$ would be negligible compared to the concentration of AlF^{+2} ion.

to as much as 100 ppm (4×10^{-3} gram atoms per liter for Mg; 2×10^{-3} for Ca) for water in areas of magnesium or calcium-bearing rocks. The $(MgF^+)/(F^-)$ ratio may thus vary from about 0.003 (0.3 per cent of the F^-) in very soft water to 0.3 (30 per cent of the F^-) in extremely hard water. The $(CaF^+)/(F^-)$ ratio may vary from < 0.0003 (0.03 per cent of F^-) to < 0.03 (3 per cent of the F^-).

To illustrate, consider the 4 hypothetical water supplies in Table III at 25° C.: A and B, very soft water at pH 5 and at pH 8, respectively; C and D, very hard water at pH 5 and 8, respectively. Assume each water is fluoridated with sodium silicofluoride to a total fluoride content of 1 ppm. As shown above, the silicofluoride will hydrolyze completely to F^- at pH 8 (samples B and D) but at pH 5 some HF is also formed (samples A and C) according to the equation:

$$\frac{(HF)}{(F^-)} = \frac{(H^+)}{K_{HF}} = \frac{10^{-5}}{6.7 \times 10^{-4}} = 0.015.$$

Some CaF^+ and MgF^+ are formed as shown above. Other metals may be ignored.

The distribution of fluoride is as follows:

TABLE III
DISTRIBUTIONS OF F IN DRINKING WATER

SAMPLE	pH	TOTAL Ca (ppm)	TOTAL Mg (ppm)	% TOTAL F AS HF	F^-	CaF^+	MgF^+
A	5	1	1	1.5	98.2	0.03	0.3
B	8	1	1	0	99.7	0.03	0.3
C	5	100	100	1.5	67.7	2.8	28
D	8	100	100	0	69.2	2.8	28

If any of these samples were ingested and absorbed into the blood stream at about pH 7.4, any HF would be converted to the F^- ion. There would be slight changes in the magnesium-bound fraction of the fluoride because of the magnesium already in the blood. Were the soft water absorbed, the magnesium-bound fraction would increase a trifle. If the hard water were absorbed, the magnesium-bound fluoride would decrease to about 5 per cent or less of the total fluoride. In all of these cases, essentially all of the fluoride in the blood stream would be in the form of the F^- ion. In no case would there be any silicofluoride either in the water ingested or in the blood stream when 1 ppm of F is added as silicofluoride in the water treatment plant.

SUMMARY

When sodium silicofluoride is dissolved in water, hydrolysis occurs; the fluoride exists in any or all of the following states: $SiF_6^=$, SiF_4, F^-, and HF. Knowing the hydrolysis constants of $SiF_6^=$ and SiF_4 and the dissociation constant of hydrogen fluoride, the distribution of fluoride in these states in a given solution may be calculated from the pH and the initial silicofluoride concentration. The hydrolysis constants of $SiF_6^=$ and SiF_4 were determined from pH measurements of silicofluoride solutions to be 7×10^{-32} and 7×10^{-26}, respectively, at 29° C.

It is concluded that in any drinking water supply with a pH of 5 or higher, fluoridated with sodium silicofluoride to the extent of 16 ppm of F or less, all of the silicofluoride is completely hydrolysed to silicic acid, fluoride ion, and hydrogen fluoride. In the water, HF constitutes only a very small fraction of the total fluoride, and when the water is ingested and absorbed into the blood stream all the fluoride is fluoride ion. There can be no question of toxicity of SiF_4 or $SiF_6^=$ under such conditions.

The stability constants of the metallo-fluorides reported by Paul have been used to show that of the many metallic ions in drinking water only calcium and magnesium are in sufficient concentration to bind at least 1 per cent of any fluoride present. When the total fluoride content is 1 ppm the percentage of fluoride bound to calcium ranges from about 0.03 per cent for very soft water to about 2.8 per cent for very hard water; the magnesium-bound fluoride ranges from 0.3 per cent for very soft water to 28 per cent for very hard water.

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FLUORINE IN TEETH OF ISRAELI CHILDREN, IN RELATION TO FLUORINE IN DOMESTIC WATERS AND TO DENTAL CARIES PREVALENCE

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SINCE the ingestion of fluorine is bound up mainly with its amounts in drinking waters, it is desirable for the public health service of every country to determine the level of fluorine in its domestic waters.[1-3]

The literature on water fluorine in relation to tooth fluorine and dental caries resistance is voluminous, and reference is made here to only a part of the more relevant investigations. Bowes and Murray[4] in England, Ockerse[5] in South Africa, Prader and Sorkin[6] in North Africa, and Harrison[7] in New Zealand showed that an increased concentration of fluorine in the drinking water goes hand-in-hand with an increased content of fluorine in the teeth. The most extensive evidence on this relation has been supplied by McClure and Likins[8] in the United States, and Schmid[9] and von Fellenberg[10] in Switzerland. Their findings emphasize strongly the relationship between increased fluorine in teeth and reduction of dental caries.

An already published survey of the fluorine content in the various drinking water supplies in Israel[11] was a basis for the present study of the fluorine content of the tooth and its correlation with the DMF index,* established in a recently completed survey of the dental caries prevalence among school children.

MATERIAL

The unusual conditions in Israel, created by the mass immigration, restricted the examination to teeth of only those children who had been born and reared in the same place. On the whole, fluorine was determined in 127 teeth of different types belonging to 114 children, 6 to 13 years old, residing in places with a water supply characterized by low (below 0.3 ppm), moderate (0.5-0.6 ppm), or high (0.6-1.3 ppm) fluorine concentration.

The teeth examined were intact shed primary teeth, and thus the portion available for analysis was the crown alone. In each case, tooth type, age, sex, race, and place of residence were noted. A record was also kept, where available, of the communal dental caries index among the children.

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*The index is expressed in terms of DMF teeth per 12- to 13-year-old Israeli-born child.

ANALYTICAL METHODS

Each tooth specimen* was completely ashed and pulverized to pass a 180-mesh sieve. Fluorine was steam-distilled from the tooth ash as hydrofluosilicic acid ($H_2Si\ F_6$), at 135° C., in the presence of perchloric acid and quartz sand, according to the method of Cremer and Voelker.[12]

In order to avoid the carrying over of perchloric acid particles by the steam into the distillate with interference of the fluorine distillation, this technic was slightly modified by attaching a distillation head to the Claisen flask

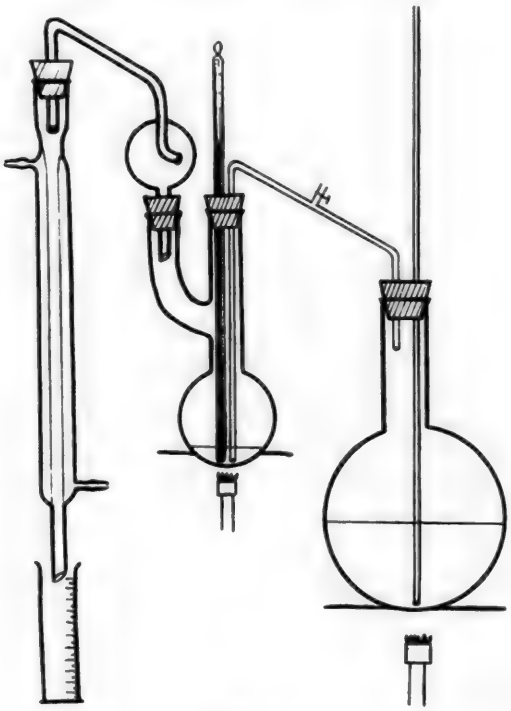

Fig. 1.

(Fig. 1). In the concentrated distillate, fluorine was determined photo-colorimetrically (Lumetron model 402-E) by the color-fading reaction of a violet iron-salicylate complex, as described by Voegtlin and Hodge.[13] The standard error of this method is not more than ±5 per cent.

RESULTS

The fluorine content of the teeth is summarized in Tables I, II, and III.

The whole material can be grouped into two categories: One contains data from Ramath-Gan, Rishon lè Zion, and Safed, representing low fluorine

*In a few instances of very small teeth, it was necessary to pool teeth of similar type from the same location.

TABLE I

FLUORINE IN SOUND PRIMARY TEETH OF CHILDREN RESIDING IN REGIONS HAVING LOW, MODERATE, AND HIGH F CONCENTRATION IN THE DRINKING WATER

LOCATION AND GEOGRAPHICAL AREA	NUMBER		FLUORINE IN DRINKING WATER (PPM)	FLUORINE CONTENT OF TEETH (MG. % OF TOOTH ASH)		
	INDIVIDUALS	SPECIMENS		MAX.	MIN.	MEAN
Ramath-Gan (near Tel Aviv coastal plain)	9	9	0.25	10.0	5.0	7.8
Rishon le Zion (southern coastal plain)	9	9	0.35	10.0	4.2	7.7
Safed (Gallilee mountains)	3	3	0.35	10.6	5.0	7.3
Tel Aviv	21	22	0.3-0.6*	37.5	11.2	21.1
Nathania (northern seashore)	7	10	0.55	33.7	15.0	23.6
Givataim, Kfar Sabah, Ramat-Hakovesh, Kiriat-Amal (coastal plain)	16	16	0.55	37.5	15.0	27.2
Jerusalem	13	14	0.5	37.5	23.7	30.3
Beth-Zera, Degania (A, B) (Jordan Valley, Kibbutzim)	6	9	0.5	35.0	18.7	24.0
Ashdot-Yaakov (Jordan Valley, Kibbutz)	6	9	0.9	27.0	20.0	24.3
Ein-Harod, Moledet, Nir-David (Yesreel Valley, Kibbutzim)	5	5	0.6-0.9	37.5	32.5	34.1
Kiriath-Chaim (Zevulun Valley)	19	21	1.3	47.5	25.0	36.5

*Water supply from different wells.

TABLE II

RELATION BETWEEN F CONTENT OF PRIMARY TEETH AND DENTAL CARIES PREVALENCE

LOCATION	MEAN F CONTENT OF TEETH (MG. %)	DENTAL CARIES PREVALENCE	
		DMF INDEX	NUMBER OF CHILDREN
Rishon le Zion	7.7	2.88	155
Ramath-Gan	7.8	2.77	548
Tel Aviv	21.1	2.45	4009
Jeusalem	30.3	1.53	1230
Degania A	22.5	1.41	91
Beth-Zera	23.0	1.37	52
Kiriath-Chaim	36.5	0.51	177

values in teeth (7.3 mg. %–7.8 mg. %); and the second group includes all other places with much higher levels of fluorine in the teeth (21.1 mg. %–36.5 mg. %). These findings of fluorine in teeth parallel the fluorine content in the drinking water; the first group having low and the second group moderate and high fluorine values in the drinking water. On the whole, no significant difference exists between regions with moderate and high fluorine content of the drinking water, although the highest mean fluorine content (36.5 mg. %) in teeth was found in Kiriath-Chaim, Zevulun Valley, where the fluorine content in water is the highest so far found in Israel (1.3 ppm).

TABLE III

COMPARISON OF FLUORINE CONTENT IN SIMILAR AND DIFFERENT TOOTH TYPES FROM THE SAME INDIVIDUALS

LOCATION AND AGE	TOOTH TYPE*	F IN TOOTH ASH (MG. %)
Tel Aviv, B. S., 11 years	V |	30.0
	| V	23.7
Beth-Zera, F. R., 7 years	| II	20.0
	I |	20.0
Degania B, B. C., 9½ years	IV |	35.0
	| III	22.5
Beth-Zera, G. J., 10 years	III |	23.7
	III |	18.7
Ashdot Yaakov, B. O., 10 years	| III	22.5
	III |	22.5
	| IV	18.8
	IV |	18.8
Nathania, C. B., 10 years	| III	28.7
	| IV	18.8
	IV |	23.7

*Types I and II, primary incisors; Type III, primary canines; Types IV and V, primary first and second molars.

There is a wide range of fluorine content in teeth of children living in the same or in different places with about the same fluorine content in the drinking water. On the other hand, no significant differences in tooth fluorine were found in places with different climatic conditions as Jerusalem and the Jordan Valley, in spite of the fact that in such hot places as the Jordan Valley, with mean annual temperature of 71.8° F., the amount of water consumed, and therefore the amount of F intake and ingestion,[14] must be significantly higher than in a much cooler area as Jerusalem, with mean annual temperature of 63.5° F. These facts are, for the time being, unexplainable.

Table II shows the inverse relationship between the fluorine content of teeth and DMF index. In areas with low fluorine content of teeth, the communal dental caries index is relatively high, going up to 2.88; whereas in areas with higher fluorine values in teeth, the dental caries index goes down to 0.51 (Kiriath-Chaim), the lowest in Israel. This confirms the findings of McClure and Likins[8] and Schmid[9] that a higher fluorine content in teeth contributes to the reduction of dental caries prevalence.

We have also had the opportunity to investigate a few cases of similar and different tooth types of the same dentition. As may be seen from Table III, sound teeth of the same individual do not always show an identical fluorine content, and the difference may be considerable. The reasons for this phenomenon are not known and it is worth while to check its relation to other causes, such as nutritional deficiencies, mineral metabolism, and systemic diseases, at an age when the particular tooth was laid and developed.

SUMMARY

One hundred and twenty-seven teeth from 114 children living in places with different drinking water supplies were analyzed for the trace element F.

A clear relationship between low fluorine content in water and teeth and a correspondingly high dental caries index, as well as the opposite, has been established.

Further investigations are needed to explain the individual differences of tooth fluorine content in children of the same region and in different teeth of the same individual.

The authors are indebted to the dentists for their cooperation in collecting teeth for analysis and to the Statistical Department of the Government of Israel for supplying the results of the dental caries survey.

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THE EVANSTON DENTAL CARIES STUDY

XV. The Caries Experience Rates of Two Groups of Evanston Children After Exposure to Fluoridated Water

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THIS paper reports the findings of the 1953 and 1954 dental examinations made on the 6- to 8- and 12- to 14-year-old continuous resident Evanston children. Comparisons are made of the data for the 1953 and 1954 groups to those of children of the same age examined in the study in 1946. The 1953 group was exposed to fluoridated water from 71 to 82 months and the 1954 group 83 to 94 months at the time of dental examinations.

METHOD

A detailed report describing the method and purpose of the study has been made previously.[1] Comparisons of the dental caries experience rates of the children examined in 1953 and 1954 are made with students of the same age in the original 1946 base line group. A schema for the study has been published.[1] It may be noted in the schema that the 1954 group of 14-year-olds includes students examined in the study for the fourth time.

They were examined in 1946, 1948, 1952, and 1954 as 6-, 8-, 12-, and 14-year-olds, respectively. All percentage figures indicating differences in rates have been subjected to statistical evaluation. Although the changes in dental caries experience rates after fluoridation are of paramount importance, other pertinent data are accumulated in the study. These data include congenitally missing teeth, oral hygiene, gingival conditions, occlusion, salivary counts of lactobacillus and the degree of fluorosis. Included in this report is the distribution of cases by lactobacillus count for the 12- to 14-year-old group for 1946, 1949, 1952, and 1954. It was the intention to include figures on fluorosis. However, in reviewing all cases from 1946-1954, the number of children with other than "normal" classification was negligible.

FINDINGS

The dental caries experience rates of the 6-, 7-, and 8-year-old children examined in 1953, after 71 to 82 months of fluoridation, indicated a reduction of approximately 57 per cent in the permanent teeth[2] (Table I). This brings

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TABLE I

DECAYED, MISSING, AND FILLED PERMANENT TEETH

YEAR	EXAMINATIONS	MONTHS OF EXPOSURE TO NaF	AGES			
			6	7	8	(6-8)
			DMF rate per hundred children			
1946	1991	None	46.85	153.49	249.93	149.76
1948	2034	12-22	23.54	103.58	194.09	92.07
1950	1244	35-46	15.33	80.93	153.52	83.26
1951	1853	47-58	12.36	66.97	161.40	80.24
1953	1457	71-82	14.73	53.35	122.49	63.52
Per cent differences 1946-1953 R = reduction I = increase			R 68.56%	R 65.24%	R 50.99%	R 57.59%
Probability of difference due to chance*			0.000	0.000	0.000	

*Any probability less than 0.05 is conventionally considered significant.

the rates of the 6- and 7-year-olds of 1953 (Fig. 1) slightly below the 1945-1946 Aurora[3] rate for children of the same age. There had been no significant change in the dental caries experience rates of the deciduous teeth until 1953 when the 6-year-olds had a reduction of borderline statistical significance[2] (Table II).

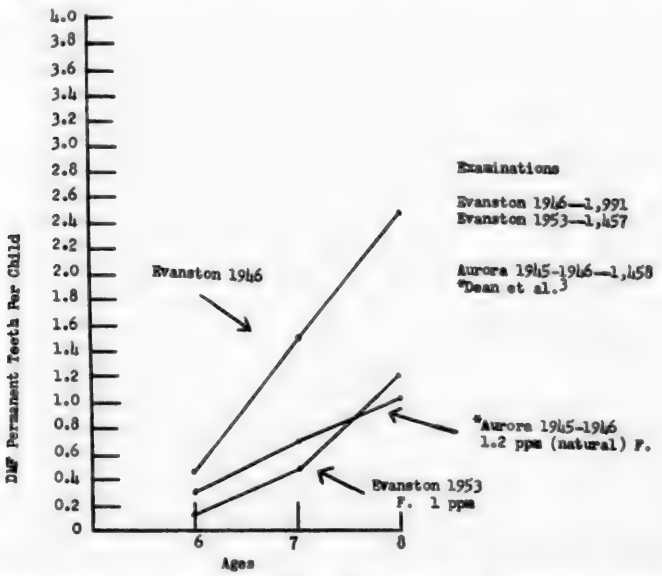

Fig. 1.—Permanent teeth dental caries experience of continuous resident Evanston and Aurora school children.

The second group of school children, the 12- to 14-year-olds, in 1952, had a reduction in dental caries experience rates associated with the permanent teeth of about 18 per cent after 59 to 70 months of fluoridation.[4] This was approximately a 4 per cent reduction for each year that the water was fluoridated. The analysis of the 1954 findings shows the reduction in decayed, missing, and filled permanent teeth per hundred children is approximately 26.59

TABLE II

DECAYED, EXTRACTED (DUE TO CARIES), AND FILLED DECIDUOUS TEETH

YEAR	EXAMINATIONS	MONTHS OF EXPOSURE TO NaF	AGES 6	7	8	(6-8)
			DEF rate per hundred children			
1946	1991	None	482.86	550.06	577.17	536.70
1948	2034	12-22	507.80	541.05	620.22	556.35
1950	1244	35-46	478.37	668.04	653.51	599.89
1951	1853	47-58	449.63	558.26	674.57	560.82
1953	1457	71-82	432.78	517.84	598.59	520.86
Per cent differences 1946-1953 R = reduction I = increase			R 10.37%	R 5.86%	I 3.71%	R 3.78%
Probability of difference due to chance*			0.05	0.12	0.26	

*Any probability less than 0.05 is conventionally considered significant.

per cent for the 12-year-olds, 24.87 per cent for the 13-year-olds, and 14.43 per cent for the 14-year-olds (Table III). The differences found here in the percentage reductions may be related to the age of these children at the time of fluoridation. It is noted that at the time of fluoridation the 12-year-olds were 5 years of age with the deciduous teeth normally in place. The crowns of the permanent teeth of these children were undergoing formation and presumably were exposed to tissue fluids containing fluorine during the pre-eruptive and posteruptive period. The 13-year-olds were 6 years of age at the time of fluoridation with the first molar teeth approximately 30 per cent erupted. The 14-year-olds at the time of fluoridation were 7 years of age with the first permanent molars in place and approximately 60 per cent of the crown portion of the central incisors erupted. It would appear that the 5-year-olds, being exposed to fluoridated water earlier in the stage of permanent tooth development, would show a greater change in caries rates than would the 7-year-olds and

TABLE III

DECAYED, MISSING, AND FILLED PERMANENT TEETH

AGE	EXAMINATIONS 12	13	14	MONTHS OF EXPOSURE TO NaF	AGE 12	13	14
Year					*DMF rate per hundred children*		
1946	418	688	595	None	763.16	1009.59	1165.88
1949	522	677	570	23-34	681.99	857.02	1086.84
1952	512	533	509	59-70	596.87	795.49	1019.84
1954	505	446	375	83-94	560.20	758.52	997.60
Per cent differences 1946-1954 R = reduction I = increase					R 26.59%	R 24.87%	R 14.43%
Probability of difference due to chance*					0.000	0.000	0.000

*Any probability less than 0.05 is conventionally considered significant.

possibly the 6-year-olds. As is noted in Table III the 12-year-olds had a difference after fluoridation of 26.59 per cent while the 14-year-olds showed a 14.43 per cent reduction and the 13-year-olds, 24.87 per cent. It is acknowledged that the 14-year-olds had: (1) 4 more teeth to consider than the 12-year-olds, (2) the teeth presumably were exposed to tissue fluids containing fluorine later

TABLE IV

DECAYED, MISSING, AND FILLED PERMANENT TEETH (RATE PER HUNDRED CHILDREN)

AGE	PUBLIC SCHOOL (WHITE CHILDREN)	PER CENT DIFFERENCES	FOSTER SCHOOL (NEGRO ONLY)	PER CENT DIFFERENCES	PAROCHIAL SCHOOL CHILDREN	PER CENT DIFFERENCES
			Evanston 1946			
No. Examinations	1226		No. Examinations 96		No. Examinations 379	
12	707.51		658.82		981.32	
13	946.17		861.76		1218.13	
14	1133.33		1035.71		1322.22	
			Evanston 1949			
No. Examinations	1251		No. Examinations 95		No. Examinations 423	
12	625.49	-10.64	697.14	+ 5.82	826.86	-15.74
13	785.89	-15.11	1127.50	+30.83	1008.38	-17.22
14	1073.31	- 6.78	1080.00	+ 4.28	1129.85	-14.55
			Evanston 1952			
No. Examinations	1041		No. Examinations 88		No. Examinations 425	
12	569.45	-19.51	476.00	-27.75	693.38	-29.34
13	746.88	-21.06	600.00	-30.38	918.02	-24.64
14	1015.27	-10.42	728.57	-29.66	1077.45	-18.51
			Evanston 1954			
No. Examinations	881		No. Examinations 79		No. Examinations 366	
12	533.87	-24.54	437.78	-33.55	651.33	-33.63
13	710.84	-24.87	744.44	-13.61	866.17	-28.89
14	960.87	-15.22	825.00	-20.34	1153.01	-12.80

in the formative and eruptive period, and (3) were in a caries exposure environment longer than the teeth of the 12- and 13-year-olds. However, comparisons are made with students of the same ages prior to fluoridation which suggest the greatest benefit of fluoridation is derived during tooth formation. It is also pointed out that there is apparently a beneficial action after crown formation which is shown to be greater during the pre-eruptive period than after eruption. From the above figures it is logical to assume that greater beneficial action from fluorine occurs in those children receiving fluorine early in the period of tooth formation.

DMF Rates: Public School White Children—Parochial School Children—Foster School (Negro) Children.—

As the control area (Oak Park) examinations included only public school white children it was necessary to evaluate the Evanston data on the basis of school groups, public white, parochial, and Foster (Negro) to make comparisons of like groups[4] (Table IV). In 1946 and 1954 the public school white children and the Foster School (Negro) children maintained nearly comparable DMF rates, while the parochial school children, although showing a definite percentage reduction in dental caries experience rates, had the highest DMF rate in 1954 of the 3 school groups. It is admitted that the Foster (Negro) school sample (79) was limited.

Occlusal surfaces with pit and fissure caries or filling: There is a decrease in the number of occlusal surfaces with pit and fissure caries or fillings when comparing the 1946 and 1954 rates (Table V). However, the rates per hundred children for the 12-, 13-, and 14-year-olds after 83 to 94 months of fluoridation are approximately the same as the 1952 rate after 59 to 70 months of fluoridation.

TABLE V

PERMANENT MOLAR OCCLUSAL SURFACES WITH PIT AND FISSURE CARIES OR FILLINGS

	EXAMINATIONS			MONTHS OF EXPOSURE TO NaF			
AGE	12	13	14		AGE 12	13	14
Year					*Rate per hundred children*		
1946	418	688	595	None	351.67	438.08	471.26
1949	522	677	570	23-34	313.41	364.99	406.49
1952	512	533	509	59-70	286.52	357.41	414.93
1954	505	466	375	83-94	286.93	354.04	416.80
Per cent differences from 1946-1954 R = reduction I = increase					R 18.41%	R 19.18%	R 11.56%
Probability of difference due to chance*					0.000	0.000	0.000

*Any probability less than 0.05 is conventionally considered significant.

Occlusal surfaces free from pit and fissure caries of fillings: Here an increase is found in the rates of the 12- and 13-year-old children from 1946 to 1954 and is quite consistent (Table VI). However, the rate in 1954 for the 14-year-old children was slightly less than the 1952 rate, but was 73.53 per cent greater than the rate for that age in the 1946 base line group. It is interesting to note that the rates in 1952 and 1954, 5 and 7 years of fluoridation, respectively, were close for all 3 ages.

TABLE VI

PERMANENT MOLAR OCCLUSAL SURFACES FREE FROM PIT AND FISSURE CARIES OR FILLINGS

AGE	EXAMINATIONS 12	13	14	MONTHS OF EXPOSURE TO NaF	AGE 12	13	14
Year					*Rate per hundred children*		
1946	418	688	595	None	194.02	190.84	183.02
1949	522	677	570	23-34	221.46	253.91	238.25
1952	512	533	509	59-70	290.42	323.26	321.02
1954	505	446	375	83-94	297.03	331.84	317.60
Per cent differences 1946-1954 I = increase R = reduction					I 53.09%	I 73.88%	I 73.53%
Probability of difference due to chance*					0.000	0.000	0.000

*Any probability less than 0.05 is conventionally considered significant.

Maxillary anterior teeth (canine to canine): The reductions found in the 1954 data, 38.08 per cent for the 12-year-olds, 34.27 per cent for the 13-year-olds, were approximately equal (Table VII). The difference associated with the 14-year-olds, considerably less than that of the 12- and 13-year-olds, was 11.94 per cent. However, the changes in 1954 were all greater than those found in 1952.

TABLE VII

DECAYED AND FILLED MAXILLARY ANTERIOR PERMANENT TEETH (CANINE TO CANINE)

AGE	EXAMINATIONS 12	13	14	MONTHS OF EXPOSURE TO NaF	AGE 12	13	14
Year					*Rate per hundred children*		
1946	418	688	595	None	127.27	157.26	172.60
1949	522	677	570	23-34	110.34	135.60	153.56
1952	512	533	509	59-70	96.29	121.01	156.97
1954	505	466	375	83-94	78.81	103.36	152.00
Per cent differences 1946-1954 R = reduction I = increase					R 38.08%	R 34.27%	R 11.94%
Probability of difference due to chance*					0.000	0.000	0.048

*Any probability less than 0.05 is conventionally considered significant.

Mandibular anterior teeth (canine to canine): The percentage reductions in decayed and filled mandibular anterior teeth continued to increase for the 12- and 13-year-olds. The 14-year-olds, although showing a 17.74 per cent difference from the 1946 rate, had a slightly higher rate in 1954 than in 1952 (Table VIII). However, it should be pointed out that rates vary from year to year and the 14-year-olds have maintained for 1949, 1952, and 1954 a rate which has varied only slightly during those years. It is also noted that the percentage differences for the 12- and 14-year-olds is not considered significant.

Permanent tooth surfaces with pre-carious lesions:* The 1954 rate was less than the rate found in 1949 and 1952 for all 3 ages (Table IX). In 1952 the rate for the 12-year-olds was greater than that found in 1949. We can find no

*Those deep pits, fissures, and grooves where a questionable etching along the peripheral border was present, or questionable soft material was demonstrable at the base of the pit, fissure or groove, and yet where there was no frank caries present.

TABLE VIII

DECAYED AND FILLED MANDIBULAR ANTERIOR PERMANENT TEETH (CANINE TO CANINE)

AGE	EXAMINATIONS 12	13	14	MONTHS OF EXPOSURE TO NaF	AGE 12	13	14
Year					*Rate per hundred children*		
1946	418	688	595	None	31.82	45.20	45.71
1949	522	677	570	23-34	28.16	35.75	38.25
1952	512	533	509	59-70	25.00	38.65	36.94
1954	505	466	375	83-94	23.76	29.82	37.60
Per cent differences 1946-1954 R = reduction I = increase					R 25.33%	R 34.03%	R 17.74%
Probability of difference due to chance*					0.082	0.010	0.136

*Any probability less than 0.05 is conventionally considered significant.

apparent reason nor can we offer a satisfactory explanation for this increase. However, the 1954 rate for the 12-year-olds is less than the 1949 rate and 57.25 per cent below the 1946 base line rate. All differences were considered significant.

TABLE IX

PERMANENT TOOTH SURFACES WITH PRE-CARIOUS LESIONS

AGE	EXAMINATIONS 12	13	14	MONTHS OF EXPOSURE TO NaF	AGE 12	13	14
Year					*Rate per hundred children*		
1946	418	688	595	None	130.62	139.10	139.33
1949	522	677	570	23-34	57.85	85.52	92.81
1952	512	533	509	59-70	81.84	68.29	91.75
1954	505	466	375	83-94	55.84	58.52	72.80
Per cent differences 1946-1954 R = reduction I = increase					R 57.25%	R 57.93%	R 47.75%
Probability of difference due to chance*					0.000	0.000	0.000

*Any probability less than 0.05 is conventionally considered significant.

Immune permanent dentitions: The 12- and 13-year-old students in 1954 had an increase of over 200 per cent in the immune permanent dentitions (Table X). The increases were considered statistically significant. In referring to immune permanent dentitions only those cases demonstrated by clinical and radiologic examination to be free from caries or fillings in the permanent teeth were included. The prescribed number, 6 anterior apical and 4 posterior bite-wing films, showing all proximal areas was necessary before the case was considered immune at the time of examination.

Decayed, missing, and filled permanent teeth expressed as per hundred teeth: The number of teeth and surfaces associated with the DMF rates from 1946 through 1954 are shown in Tables XI and XII. It was felt necessary to express rates in terms of surfaces to total surfaces as multiple carious lesions and fillings are less likely to be obscured.[4] However, all 3 methods, namely; per hundred children, per hundred teeth, and per hundred surfaces, all express approximately the same proportion of percentage differences in rates.

TABLE X

IMMUNE PERMANENT DENTITIONS

AGE	EXAMINATIONS 12	13	14	MONTHS OF EXPOSURE TO NaF	AGE 12	13	14
Year					*Rate per hundred children*		
1946	418	688	595	None	1.44	1.02	1.01
1949	522	677	570	23-34	1.53	2.51	1.75
1952	512	533	509	59-70	2.73	2.44	0.39
1954	505	466	375	83-94	4.55	3.36	1.87
Per cent differences 1946-1954 R = reduction I = increase					I 215.97%	I 229.41%	I 85.15%
Probability of difference due to chance*					0.002	0.003	0.129

*Any probability less than 0.05 is conventionally considered significant.

TABLE XI

DECAYED, MISSING, AND FILLED PERMANENT TEETH (RATE PER HUNDRED TEETH)

YEAR	NUMBER OF EXAMINATIONS	TEETH PRESENT	TEETH EXTRACTED	TOTAL NUMBER TEETH CONSIDERED	DMF RATE PER HUNDRED TEETH	DIFFERENCES FROM 1946 (%)
12-Year-Olds						
1946	414	9758	45	9803	32.00	
1949	627	14612	51	14663	29.41	- 8.09
1952	516	11923	36	11959	25.60	-20.00
1954	509	11874	17	11891	24.39*	-23.78
13-Year-Olds						
1946	692	17988	118	18106	38.47	
1949	641	16469	77	16546	35.77	- 7.08
1952	537	13868	33	13901	30.69	-20.22
1954	448	11632	33	11665	29.16*	-24.20
14-Year-Olds						
1946	617	16283	129	16412	43.24	
1949	503	13351	105	13456	40.38	- 6.11
1952	506	13685	61	13746	37.63	-12.97
1954	375	10117	58	10175	36.50*	-15.58

*Difference between 1946 and 1954 rate is significant.

TABLE XII

DECAYED, MISSING, AND FILLED TOOTH SURFACES (RATE PER HUNDRED SURFACES)

YEAR	NUMBER OF EXAMINATIONS	TEETH PRESENT	TEETH EXTRACTED	TOTAL TOOTH SURFACES CONSIDERED	DMF RATE PER HUNDRED SURFACES	DIFFERENCES FROM 1946 (%)
12-Year-Olds						
1946	414	9758	45	49015	11.42	
1949	627	14612	51	73315	10.53	- 7.79
1952	516	11923	36	59795	9.01	-21.10
1954	509	11874	17	59455	8.09*	-29.16
13-Year-Olds						
1946	692	17988	118	90530	13.82	
1949	641	16469	77	82730	13.04	- 5.64
1952	537	13868	33	69505	10.92	-20.98
1954	448	11632	33	58352	10.33*	-25.31
14-Year-Olds						
1946	617	16283	129	82060	15.92	
1949	503	13351	105	67280	14.82	- 6.85
1952	506	13685	61	68730	13.94	-12.44
1954	375	10117	58	50875	13.09*	-17.78

*Difference between 1946 and 1954 rate is significant.

Comparison with Aurora: In comparing the Evanston DMF rate per child with that of Aurora[3] (Fig. 2), a considerable difference is noted between the two cities. However, it has been anticipated that it would be at least 10 years post-fluoridation, or approximately 1957, before the Evanston rate will closely approach the Aurora rate for this age group.

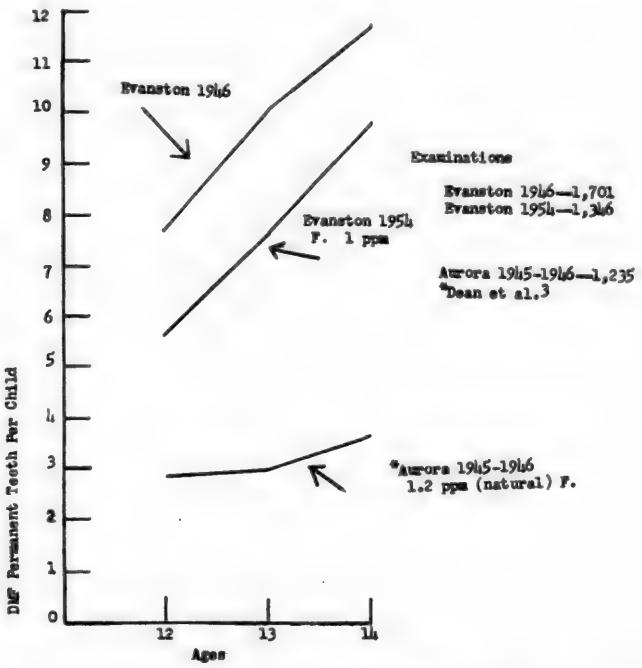

Fig. 2.—Permanent teeth dental caries experience of continuous resident Evanston and Aurora school children.

Lactobacillus count—distribution of cases in the low, moderate, and high lactobacillus count divisions: Saliva specimens for lactobacillus counts were taken on 10 per cent of the students in the study. These specimens were obtained only from continuous resident students. A more detailed report on this procedure has been given.[5] The distribution of cases is seen in Table XIII. A decrease of cases is seen in the high count group and is consistent in 1952 and 1954. There is, however, a noticeable fluctuation in the low and moderate groups from year to year. It has been suggested that lactobacilli are indicators of conditions favorable for the carious process to exist. Therefore, the fact that there are fewer cases found in the high count group and a reduction in dental caries in the 1954 group may indicate that, after fluoridation, conditions are altered and as such are not as favorable to the growth of lactobacillus or to the progress of caries.

Summary of changes associated with various rate counts: A listing of reductions found in dental caries experience rates is seen in Table XIV. It is

TABLE XIII

DISTRIBUTION OF CASES IN LOW, MODERATE, AND HIGH LACTOBACILLUS COUNT DIVISIONS

	LOW (L* PER ML., 0 TO 9999)	MODERATE (L PER ML., 10,000 TO 49,999)	HIGH (L PER ML., 50,000 AND OVER)
	(%)	(%)	(%)
1946	61.60	29.91	8.49
1949	55.29	36.40	8.31
1952	46.57	47.65	5.78
1954	68.91	27.70	3.39

*L = lactobacillus.

TABLE XIV

DIFFERENCES IN THE 1946 AND 1954 DENTAL CARIES EXPERIENCE RATES (BASED ON RATES PER HUNDRED CHILDREN UNLESS OTHERWISE INDICATED)

	AGE		
	12	13	14
	(%)	(%)	(%)
DMF permanent teeth	*R 26.59	R 24.87	R 14.43
Permanent molar occlusal surfaces with pit and fissure caries or fillings	R 18.41	R 19.18	R 11.56
Permanent molar occlusal surfaces free from pit and fissure caries or fillings	†I 53.09	I 73.88	I 73.53
Maxillary anterior permanent teeth decayed and filled (canine to canine)	R 38.08	R 34.27	R 11.94‡
Mandibular anterior permanent teeth decayed and filled (canine to canine)	R 25.33‡	R 34.03	R 17.74‡
Pre-carious lesions on permanent tooth surfaces	R 57.25	R 57.93	R 47.75
Immune permanent dentitions	I 215.97	I 229.41	I 85.15‡
Following differences based on rates per hundred teeth and per hundred surfaces			
Teeth	R 23.78	R 24.20	R 15.58
Surfaces	R 29.16	R 25.31	R 17.78

*R = reduction.
†I = increase.
‡Statistically not significant.

noted that the 14-year-olds apparently trail the 12- and 13-year-old students in percentage changes in all categories listed except that concerning occlusal surfaces free from pit and fissure caries or fillings. Here both the 13- and 14-year-olds have approximately the same difference (73 per cent) while the 12-year-olds have 53 per cent. As the 13- and 14-year-olds probably had more time for the occlusal surfaces to become carious than the 12-year-olds it is interesting to note that a greater number are apparently remaining free.

The greatest difference, expressed in terms of per cent, is an increase of 229.41 per cent in children free of all dental caries or fillings in all permanent teeth of the 13-year-old group. When expressed in terms of per hundred children there were 1.02 cases in 1946 while in 1954 the rate rose to 3.36. This difference is statistically significant. The 12-year-old group of 1954 showed an increase of 215.97 per cent in cases immune to dental caries at the time of dental examination. The difference between the 1946 and the 1954 rate in

terms of per hundred children is statistically significant. The increase in immune cases for the 14-year-old children in 1954 in terms of percentage is 85.15. However the difference in cases per hundred children in 1954 when compared with the rate in 1946 is not statistically significant.

DISCUSSION

It should be noted that the 6-year-olds, in 1953, showed a reduction in the dental caries experience rates of the deciduous teeth. This change is considered to be of borderline significance as the probability of difference due to chance is only 0.05. Any probability less than 0.05 is conventionally considered significant. This reduction associated with the deciduous teeth of the 6-year-olds may be related to two somewhat simultaneous happenings, (1) the birth dates of these children, and (2) the date fluorine was added to the Evanston water. Approximately $\frac{5}{12}$ of the 6-year-old children examined in 1953 were born after fluorine was added to the water on Feb. 11, 1947. The mothers of one half of these children were using fluoridated water prior to conception. The remainder ranged from the first to the sixth month of pregnancy at the time sodium fluoride was placed in the Evanston water. It follows that the deciduous teeth of the 6-year-old group were formed, erupted, and matured under the influence of the F ion. Therefore, it seems logical to assume that the deciduous teeth of the 6-year-olds have had a greater protection against caries than those of the 7- and 8-year-old groups.

We are often asked if the use of fluoridated water is beneficial when ingested after tooth eruption. This may be answered at least partially by referring to the work with rodents, and diets by Shaw and Sognnaes.[6] They point out that fully formed teeth in active function are not completely devoid of ionic exchange. They also point out that in the ionic exchange of the erupting tooth there appears to be a much greater ability to take up and turn over certain ions than a tooth that has been in the oral cavity for a longer period of time.

SUMMARY

1. The permanent teeth dental caries experience rate of the 6- to 8-year-old children was reduced 57.59 per cent after 71 to 82 months of fluoridation.

2. The 6-year-old children showed a reduction in caries rates of the deciduous teeth considered to be border line from a statistical viewpoint. The change in rates of the deciduous teeth of the 7- and 8-year-olds is not significant.

3. The 12- to 14-year-old children after 83-94 months of fluoridation showed a reduction of approximately 21.96 per cent in dental caries experience rates of the permanent teeth.

4. The reductions in rates shown in the maxillary anterior permanent teeth of the 12-, 13-, and 14-year-olds were considered significant. The differences in rates concerning the mandibular anterior teeth were not significant.

5. The permanent tooth surfaces with pre-carious lesions (per hundred children) in 1954 were approximately one half the number found in 1946.

6. The immune permanent dentitions increased over 200 per cent for the 12- and 13-year-olds. These increases are considered significant. The 85.15 per cent increase for the 14-year-olds is not significant.

7. The percentage change associated with the caries rate of the permanent teeth are only slightly dissimilar when expressed either as per hundred children, per hundred teeth, or per hundred surfaces.

8. Distribution of cases concerning salivary lactobacillus counts continue to be somewhat fluid in the low and moderate divisions but has shown a steady lowering of cases in the high count group.

9. The Evanston 6- and 7-year-olds of 1953 have a lower dental caries experience rate after 71 to 82 months of fluoridation than the Aurora 6- and 7-year-olds of 1945-1946 with lifetime exposure to water naturally fluoridated to 1.2 ppm.

10. It is anticipated that it will be at least 1957 before the Evanston rate of the 12- to 14-year-old group will approximate the Aurora rate for the same age children.

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EXPERIMENTAL CARIOSTASIS IN RATS ON A TEA DIET OF HIGH FLUORINE CONTENT

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THE average daily human diet contains from 0.25 to 0.30 mg. of fluorine,[1] normally insufficient for effective control of dental caries. For this reason, water supplements of fluorine have been found desirable.[2, 3] In the absence of a communal water supply, caries can be retarded either by direct application of fluorine compounds to the teeth,[4] by dietary supplements of fluorine salts,[5, 6] or by consumption of foods with high fluoride content, notably sea foods or tea; but the availability of dietary fluorine in contrast to that in water still seems to be in question.

To ascertain the concentration of fluorides in food necessary for effective cariostasis, we completed a number of experiments using instant tea powder and a combination of tea and camellia leaves, both foods with high fluorine concentrations.

Standard Brands instant tea supplies fluorine in concentrations greater than 170 ppm. Camellia, a hardy species of the tea genus, also was used because of its high fluorine content. Camellia plants were grown on one part Lansdale silt loam and one part Tennessee brown rock phosphate containing 3.7 per cent fluorine. Fluorine concentrations in the dried leaves of the camellia averaged 2,000 ppm.

METHOD

Twenty 21-day-old Wistar rats were paired according to litter, sex, and weight. One rat from each 10 pairs was placed on a diet containing 20 per cent instant tea by weight. Dietary intake of fluorine was 35 ppm. The remaining 10 rats were fed a control diet (Table I). Weights of the individual rats at 21 days averaged 37 Gm.

RESULTS

The first death occurred on the fortieth day of the experiment in a rat on the tea diet. Accordingly the remaining animals were sacrificed. The number of cavities were evaluated in the individual rats by direct inspection under low-power magnification and by x-ray examinations. No caries were found in the rats on the tea diet whereas the animals on the control diet averaged 1.5 carious teeth per rat. The fluorine content of femurs in the control group averaged 0.0165 per cent compared with 0.170 per cent (10 times greater) in

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the femurs of the animals on the tea diet. The fluorine content of the tea and femurs was evaluated by the method of McClendon and Foster.[7] At death, the rats on the tea diet averaged 55 Gm. in weight in contrast to 92 Gm. for the rats on the control diet.

TABLE I

CONTENTS OF CONTROL AND EXPERIMENTAL DIETS OF TEA AND TEA-CAMELLIA LEAVES

CONTROL DIET*	GRAMS	TEA DIET†	GRAMS	TEA-CAMELLIA DIET‡	GRAMS
Yellow corn (freshly ground)	73.0	Instant tea§	20.0	Instant tea§	5.0
Linseed meal	3.0	Yellow corn (freshly ground)	53.0	Camellia leaves	5.0
Corn oil	4.0			Yellow corn (freshly ground)	63.0
Liver powder	3.0				
Sucrose	12.0	Other constituents same as in control diet		Other constituents same as in control diet	
Active dry yeast§	1.0				
L lysine	0.5				
DL tryptophan	0.5				
DL methionine	0.5				
Sodium chloride	1.0				
Niacin	0.005				
Alfalfa meal	1.5				

*Fluorine content, 1 ppm.
†Fluorine content, 35 ppm.
‡Fluorine content, 110 ppm.
§Standard Brands.

A tea-camellia diet was then investigated for 2 reasons: to reduce caffeine intake, and to raise the dietary fluorine concentration. Ten pairs of rats were selected, each 21 days old, averaging 38 Gm. in weight. As before, one of each pair was fed the control diet and the other a diet containing 5 per cent instant tea and 5 per cent powdered camellia leaves by weight. Dietary intake of fluorine was 110 ppm (Table I). At 112 days the experiment was terminated. The rats on the control diet averaged 102 Gm. in weight with 4.6 carious teeth each. Those on the tea-camellia diet averaged 81 Gm. in weight with 1.5 carious teeth per rat.

DISCUSSION

Tea and tea-camellia diets containing high concentrations of dietary fluorine evidently have a cariostatic effect on the rat. Of the two, the tea-camellia diet contains 3 times as much fluorine and is less toxic.

The marked differences in weight at death between the animals on the experimental diet and those on the control diet are probably due to the caffeine toxicity and low calorie intake.

Under experimental conditions, the rat requires relatively high concentrations of fluorine for effective cariostasis.[6] Inhibition of dental caries in the human being may be expected with comparatively lower concentrations of fluorine in natural food than is required for the rat. Among natural foods, only tea and sea food contain higher than average dietary concentrations of fluorine. Sea foods are not regularly consumed by most human beings in quantities sufficient for effective cariostasis. Fluorine concentration in tea infusions is not sufficiently high to retard caries in experimental animals,[8] but

for human beings the concentration would seem adequate. Therefore, it is suggested that where caries is prevalent in tea drinking populations, it results from insufficient quantities of tea infusion in the daily diet or from the presence of other cariogenic factors.

SUMMARY

Fluorine in tea or camellia leaves supplied in the diet of Wistar rats was found to be cariostatic. This suggests that dietary fluorine can be cariostatic if present in sufficient concentrations.

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A COMPARISON OF SALIVARY PROTEASE ACTIVITY IN THE HUNT-HOPPERT CARIES-RESISTANT AND CARIES-SUSCEPTIBLE RATS

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HUNT and Hoppert[1-6] developed 2 strains of rats, one caries-susceptible and the other caries-resistant. The susceptible animals show dental caries at approximately 70 days of age and the resistant ones at about 585 days.

Rosen, Benarde, Hunt, and Hoppert[7] investigating possible differences in the oral microflora of the 2 strains found that lactobacilli and *Streptococcus salivarius* were recovered more frequently from caries-susceptible than from caries-resistant rats. Rosen, Benarde, Fabian, Hunt and Hoppert[8] found that the saliva of susceptible animals had a lower relative viscosity than that of resistant animals. Subsequently, this investigation was extended to the study of enzymes occuring in the saliva of these strains. A preliminary study showed that the widest difference occurred with protease.

Sreebny, Meyer, and Bachman[9] found that submaxillary gland extracts of Hunt-Hoppert caries-susceptible male rats had approximately 5 times as much protease activity as resistant males. The object of this study was to determine whether these differences in protease activity also existed in the saliva of these animals. Both male and female animals were included in this study in order to determine the possible influence of sex on the protease activity of the saliva.

EXPERIMENTAL METHODS

The method of Benarde, Fabian, Rosen, Hunt, and Hoppert[10] was used to collect rat saliva. This involves anesthesia by intraperitoneal injection of Nembutal followed by a small dose of pilocarpine given subcutaneously. Prior to injection with pilocarpine, the teeth and oral cavity were vigorously brushed and cleaned with a stencil brush dipped in distilled water so as to reduce contamination by fecal matter, sawdust, and retained food materials. Saliva was tested from individual rats whenever possible, but when pooled salivas were used, they were collected from rats of the same litter. All determinations were begun within 1 hour after collection of the saliva.

Protease determinations were carried out by modifying the casein-digestion method of Kunitz and co-workers.[11] One per cent casein was prepared

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by dissolving Hammersten casein in a 0.2M borate buffer. The solution was then heated in a water bath at 95° to 98° C. for 10 minutes to inactivate any enzymes present in the casein.

This solution was stable for at least 3 weeks when kept at 4° C. Equal amounts of saliva and substrate, usually 1 ml., were mixed and incubated for 4 hours at 37° C. One-half the volume was withdrawn immediately after mixing and added to 3 volumes of 5 per cent trichloracetic acid for a zero time reading. The precipitate formed was filtered through No. 40 filter paper after standing at room temperature for 1 hour. At the end of the incubation period the above procedure was repeated with the remaining half of the saliva-substrate mixture.

The concentration of split products in the filtrate, measured as microgram of tyrosine liberated per milliliter of saliva, was determined colorimetrically at 600 mμ by Herriot's[12] method using a Hellige colorimeter or a Bausch and Lomb "Spectronic 20." Standard curves were constructed using various concentrations of L-tyrosine.* Controls using casein and buffer were included in each experimental trial to eliminate the possibility of nonenzymatic breakdown of the substrate.

RESULTS

The results of the first experiment involving protease activity in saliva are listed in Tables I and II. This group of animals was 88 to 125 days of age. The average amount of tyrosine liberated per milliliter of saliva by enzymatic action from saliva of resistant males was 58.2 ± 9.3 μg, whereas the average value for saliva from susceptible males was 178.2 ± 18.1 μg. Thus, the susceptible males showed an activity 3.1 times that of resistant males. The t value for this comparison was 5.9, which is significant at the 0.1 per cent level. Similarly, a smaller difference was obtained when comparing susceptible with resistant females. The susceptible females showed 2.4 times the activity of resistant females. The average value for susceptible females was 126.4 ± 18.0, whereas the average value for resistant females was 53.6 ± 6.5. The t value for this comparison was 3.8, which is significant at the 0.1 per cent level. Susceptible males showed greater activity than females, but there were no differences between sexes of resistant animals.

The second experiment was used to verify the results of the first. Previously untested rats 125 to 140 days of age were used in this phase. The choice of age group had no particular significance at the time they were tested. The results as listed in Tables III and IV again brought out significant differences between resistant and susceptible strains of both sexes. As in the previous experiment, susceptible males and females showed 2.6 times the activity of resistant males and females, respectively. However, significant differences between sexes of the same strain were not obtained. It should be pointed out that the average values for the 125- to 140-day-old rats were higher in all cases than those obtained with 88- to 125-day-old animals, suggesting an increase in activity with age.

*Obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio.

In order to determine whether protease activity was a factor in causing caries, it was decided to determine protease activity at the pre-caries level. The animals selected were from 49 to 55 days of age. This experiment was extended to measure the enzyme activity in the saliva of the same rats at a later age.

TABLE I

PROTEASE ACTIVITY OF SALIVA OF CARIES-RESISTANT AND CARIES-SUSCEPTIBLE RATS 88-125 DAYS OF AGE EXPRESSED AS MICROGRAM OF TYROSINE LIBERATED PER MILLILITER OF SALIVA

TRIAL NO.	RESISTANT MALES	RESISTANT FEMALES	SUSCEPTIBLE MALES	SUSCEPTIBLE FEMALES
1	12.0	38.0	212.0	96.0
2	34.0	96.0	258.0	144.0
3	92.0	58.0	122.0	192.0
4	86.0	33.0	98.0	196.0
5	42.0	28.0	262.0	156.0
6	100.0	40.0	138.0	118.0
7	40.0	62.0	144.0	124.0
8	52.0	66.0	180.0	68.0
9	42.0	48.0	148.0	208.0
10	82.0	67.0	220.0	24.0
11	---	---	---	64.0
Average and Standard error	58.2 ± 9.3	53.6 ± 6.5	178.2 ± 18.1	126.4 ± 18.0

TABLE II

SIGNIFICANCE OF RESULTS ON PROTEASE ACTIVITY OBTAINED FROM RATS 88-125 DAYS OF AGE

COMPARISON	t VALUE	LEVEL OF SIGNIFICANCE (PER CENT)
S♂ with R♂	5.9	0.1
S♀ with R♀	3.8	0.1
R♂ with R♀	0.40	None
S♂ with S♀	2.0	5.0

TABLE III

PROTEASE ACTIVITY OF SALIVA OF CARIES-RESISTANT AND CARIES-SUSCEPTIBLE RATS 125-140 DAYS OF AGE EXPRESSED AS MICROGRAM OF TYROSINE LIBERATED PER MILLILITER OF SALIVA

TRIAL NO.	RESISTANT MALES	RESISTANT FEMALES	SUSCEPTIBLE MALES	SUSCEPTIBLE FEMALES
1	124.0	72.0	270.0	194.0
2	142.0	82.0	140.0	100.0
3	30.0	72.0	284.0	100.0
4	142.0	126.0	164.0	108.0
5	144.0	162.0	196.0	214.0
6	62.0	28.0	344.0	308.0
7	50.0	62.0	268.0	336.0
8	66.0	---	272.0	416.0
Average and Standard error	95.0 ± 16.8	86.3 ± 16.7	242.2 ± 24.3	220.0 ± 42.6

TABLE IV

SIGNIFICANCE OF RESULTS ON PROTEASE ACTIVITY OBTAINED FROM RATS 125-140 DAYS OF AGE

COMPARISON	t VALUE	LEVEL OF SIGNIFICANCE (PER CENT)
S♂ with R♂	5.0	0.1
S♀ with R♀	2.9	0.1
R♂ with R♀	0.37	None
S♂ with S♀	0.45	None

From the results in Tables V and VI, it can be seen that with rats only 49 to 55 days old, the salivary protease activity for susceptible males was 1.6 times that of resistant males. The t value for this comparison was 3.7, which is significant at the 0.1 per cent level. Too much emphasis should not be placed on this difference as the average values obtained for protease activity were very low, and resistant females showed almost as much activity as susceptible males, whereas in all other experiments there was always a wide difference between these two groups. Significant differences were not obtained between susceptible and resistant females. In addition, there were no differences between sexes of the same strain in this experiment.

TABLE V
PROTEASE ACTIVITY OF SALIVA OF CARIES-RESISTANT AND CARIES-SUSCEPTIBLE RATS 49-55 DAYS OF AGE EXPRESSED AS MICROGRAM OF TYROSINE LIBERATED PER MILLILITER OF SALIVA

TRIAL NO.	RESISTANT MALES	RESISTANT FEMALES	SUSCEPTIBLE MALES	SUSCEPTIBLE FEMALES
1	20.0	268.0	52.0	84.0
2	28.0	26.0	124.0	68.0
3	46.0	140.0	80.0	50.0
4	28.0	43.0	80.0	64.0
5	54.0	38.0	76.0	64.0
6	46.0	42.0	54.0	72.0
7	48.0	66.0	72.0	0.0
8	54.0	32.0	40.0	70.0
9	58.0	44.0	46.0	82.0
10	42.0	50.0	36.0	154.0
11	42.0	44.0	81.0	0.0
12	42.0	48.0	60.0	55.0
13	44.0	38.0	54.0	68.0
14	28.0	42.0	76.0	48.0
15	---	56.0	---	---
16	---	42.0	---	---
Average and Standard error	41.4 ± 3.0	63.7 ± 15.1	66.5 ± 6.1	62.8 ± 9.9

TABLE VI
SIGNIFICANCE OF RESULTS ON PROTEASE ACTIVITY OBTAINED FROM RATS 49-55 DAYS OF AGE

COMPARISON	t VALUE	LEVEL OF SIGNIFICANCE (PER CENT)
S♂ with R♂	3.7	0.1
S♀ with R♀	0.05	None
R♂ with R♀	1.4	None
S♂ with S♀	0.32	None

The rats used in the above experiment were tested again for salivary protease activity when they were 88 to 92 days of age. Tables VII and VIII show that susceptible males had 3.2 times the activity of resistant males, and that susceptible females had about 2.1 the activity of resistant females. Significant differences were not found between sexes of resistant animals. However, there was a difference in activity between male and female susceptible animals which confirmed the results obtained in the 88- to 125-day-old group. It should be noted in Table VII that the average protease activity of susceptible rats of the 88- to 92-day-old group increased at least twofold over that of the 49- to 55-day-old animals. In the same period there were only slight changes in the proteolytic activity of the resistant animals.

TABLE VII

PROTEASE ACTIVITY OF SALIVA OF CARIES-RESISTANT AND CARIES-SUSCEPTIBLE RATS 88-92 DAYS OF AGE EXPRESSED AS MICROGRAM OF TYROSINE LIBERATED PER MILLILITER OF SALIVA

TRIAL NO.	RESISTANT MALES	RESISTANT FEMALES	SUSCEPTIBLE MALES	SUSCEPTIBLE FEMALES
1	62.0	70.0	254.0	236.0
2	47.0	76.0	268.0	206.0
3	80.0	76.0	132.0	186.0
4	34.0	54.0	136.0	132.0
5	42.0	38.0	164.0	60.0
6	81.0	47.0	192.0	49.0
7	44.0	48.0	96.0	100.0
8	38.0	54.0	140.0	130.0
9	76.0	44.0	176.0	100.0
10	62.0	38.0	280.0	56.0
11	52.0	40.0	220.0	66.0
12	54.0	42.0	196.0	40.0
13	---	28.0	136.0	128.0
14	---	96.0	120.0	---
15	---	48.0	170.0	---
Average and Standard error	56.0 ± 4.7	53.3 ± 4.8	178.7 ± 14.5	114.5 ± 17.5

TABLE VIII

SIGNIFICANCE OF RESULTS ON PROTEASE ACTIVITY OBTAINED FROM RATS 88-92 DAYS OF AGE

COMPARISON	t VALUE	LEVEL OF SIGNIFICANCE (PER CENT)
S♂ with R♂	8.1	0.1
S♀ with R♀	3.4	0.1
R♂ with R♀	0.40	None
S♂ with S♀	2.8	1.0

DISCUSSION

One reason for the present investigation was that Sreebny, Meyer, and Bachman[9] had previously shown that submaxillary gland extracts of Hunt-Hoppert susceptible male rats had 5½ times the protease activity found in the glands of resistant males. The results of the present work extend this observation not only by showing that similar differences exist in the saliva, but that significant differences also occur between resistant and susceptible females.

Several interesting parallels may be drawn to an earlier investigation carried out by Sreebny, Meyer, and Bachman[13] comparing submaxillary protease activity between sexes of Sprague-Dawley rats. They noted that males exhibited more activity than females, but that these differences did not occur until after puberty and that activity increased with age. In this investigation, Susceptible males also exhibited more activity than susceptible females. Moreover, the protease activity was slight until after puberty and protease activity increased with age.

The differences in protease activity between susceptible and resistant rats should be considered from two aspects: why do they exist, and what is their relationship, if any, to caries? A possible causal relation between protease activity and caries is suggested because the average caries time in the susceptible line and the ages at which the differences in protease activity appear approximate each other rather closely.

The data suggest that protease activity may not contribute too much to the initiation of caries. The average caries time for susceptible animals is about 70 days. However, this is the time at which gross lesions in the teeth are detected. In reality, the carious lesion starts earlier. If protease activity is a major factor in the cause of caries, the differences obtained at the 49 to 55 day age level should have been greater in the case of the males and also should have appeared in the females. On the contrary, the values obtained did not show differences of significant magnitude until the animals were about 90 days old. Intervening examinations were not made. By the time the animals were 90 days old, the susceptible rats had already developed caries. These facts, however, do not preclude the possibility that protease may accelerate the carious process since protease activity increases sharply as susceptible rats develop caries.

Protease may accelerate caries in several ways. The enzyme may act on the small fraction of protein in the enamel. It may also attack dentinal protein after the initial lesion has been established. It has been observed in the Hunt-Hoppert rats that caries develops rapidly in susceptible animals after the initial lesion, whereas caries develops slowly in resistants. Another way in which protease may accelerate caries is in the breakdown of proteins in the mouth to provide amino acids for cariogenic bacteria.

It is possible to theorize that since this study dealt with highly inbred lines, differences in protease activity might reflect only the results of the inbreeding. There are at least 3 conceivable explanations for the relationship between protease content of the saliva and susceptibility to caries, other than the theory that protease is one of the causes of caries: (1) Both the amount of protease and the high degree of susceptibility to caries might, conceivably, be 2 effects of the same gene in the susceptible strain. (2) The gene for protease content might be closely linked (on the same chromosome) with the gene, or one of the principal genes, for caries susceptibility. (3) A more probable hypothesis is that the protease content and the degree of susceptibility are independent traits which have been fixed by the process of inbreeding. One of the important effects of inbreeding is to render the stock more and more homozygous for all genes. The alleles which become homozygous depend on those which were present in the foundation stock and on the chance unions of gametes. It is a commonplace fact in genetics that the presence of 2 traits in a highly inbred strain is a result of the inbreeding process itself and not due to the situation that one of the traits is a cause of the other. Thus, the high protease content of the saliva may not be one of the causes of the extreme susceptibility to caries in our susceptible line.

SUMMARY

A study was made to compare the protease activities of the saliva of the Hunt-Hoppert caries-resistant and caries-susceptible rats.

In mature animals, the saliva of susceptible males displayed from 2.6 to 3.2 times as much protease activity as that of resistant males. Mature susceptible females showed 2.1 to 2.6 times the activity of resistant females.

Susceptible males exhibited slightly greater activity than susceptible females in rats 88 to 125 days old. However, these differences were not observed in rats prior to puberty or in rats older than 125 days.

Activity increased sharply with age in the case of susceptible animals, whereas much smaller changes were found in resistants of comparable ages.

The possible relationship of protease activity to caries was discussed.

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THE CHLORIDE LEVEL OF SALIVA. II.

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AS REPORTED previously, the chloride level of a group of 251 children was determined and the results estimated were found significant at the 99 per cent level of confidence when comparing the chloride levels of the salivas of the children with no decay, against those with moderate and extensive odontoclasia.

Since that time, two further groups of children have been tested and the results obtained verified those arrived at in the primary study. The tests were conducted in the same way as for the first series and the examinations and classification for caries incidence were also continued in the same manner as reported in the earlier paper. The following conditions were continued to be observed: (a) A morning appointment was made for securing saliva, (b) the subject was at least 2 hours away from food—solid or liquid, and (c) no artificial stimulation was used .

RESULTS

Results of this study are shown in Tables I and II.

TABLE I

SERIES	NO.	AVERAGE CHLORIDE VALUE
I	251	15.81 ± 4.32 meq./L.
II	231	16.08 ± 4.85 meq./L.
III	259	16.61 ± 4.68 meq./L.
I, II, III	741	16.17 ± 4.63 meq./L.

Some of the children tested were in all the three series and some of them in two series. Hence, the 741 cases in series I, II, and III contain numerous replicate children. The total number of individuals seen, not counting them as replicates, was 431 and their mean chloride value was 16.25 ± 4.63 meq. per liter.

In considering the children that were tested in replicate tests—spaced at 6-month intervals—an interesting observation was made. One hundred and nine children were seen 3 times at 6-month intervals and when their test results were examined, the following conditions were found to exist:

23 cases had no replicates within ± 0.5 meq./L. of each other,
65 cases had 2 replicates within ± 0.5 meq./L. of each other, and
21 cases had 3 replicates within ± 0.5 meq./L. of each other.

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TABLE II

	SERIES I			SERIES II			SERIES III		
	NO.	MEAN	SIGMA	NO.	MEAN	SIGMA	NO.	MEAN	SIGMA
A 1 Caries free	30	12.59 ± 2.69 meq./L.		21	14.32 ± 2.65 meq./L.		22	14.15 ± 2.67 meq./L.	
B 2 Moderate static	11	13.02 ± 3.06		15	13.36 ± 2.05		19	15.50 ± 4.06	
3 Moderate active slow	74	14.02 ± 2.70		87	14.67 ± 3.22		85	15.16 ± 3.68	
4 Moderate active rapid	41	17.12 ± 3.70		32	17.49 ± 5.28		19	18.03 ± 5.31	
C 5 Extensive static	2	12.83							
6 Extensive active slow	12	16.34 ± 2.81		30	16.03 ± 4.74		44	17.50 ± 5.14	
7 Extensive active rapid	81	18.35 ± 4.84		46	19.47 ± 6.29		70	18.52 ± 4.91	

Of the 23 cases with no replicates within ± 0.5 meq. per liter of each other, only 8 (34.8 per cent) were found to be constant in regard to their dental decay activity rate for that 12-month period. Of the 21 cases with 3 replicates, however, 18 (85.71 per cent) were found to be constant in regard to their dental decay activity rate for the same 12-month period.

In regard to reduplicability, 86 cases (79 per cent) of the 109 cases tested had at least 2 of their three replicate tests, in the 12-month period, within ± 0.5 meq. per liter of one another.

SUMMARY

For 3 series of children, spread over 18 months, the chloride level of the saliva was tested, and it was established that there is a constant significant relationship between that level and the degree of caries activity. The higher the level of a child's salivary chloride, the greater is the tendency for dental decay activity. The mean average for these children was established at 16.25 ± 4.63 meq. per liter with a range of 6.49 to 42.87 meq. per liter. It was found that replicability was at about the 80 per cent level for 2 out of 3 tests conducted at 6-month intervals.

EXPERIMENTAL OBSERVATIONS ON MELANOIDIN FORMATION IN HUMAN CARIOUS TEETH

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BROWNING of the organic matrix is an integral part of the caries process in human teeth. The pigment thus formed is extremely insoluble in the usual solvents and is limited to the organic fraction of the involved tooth structure. Deakins[1] isolated the pigment from naturally occurring carious lesions and identified it as a melanoidin on the basis of its chemical composition. In a series of in vitro studies in this laboratory,[2-5] the pigment was produced synthetically in noncarious human teeth by methods which involved the interaction between degradation products of glucose and the protein component of the organic matrix. This finding was subsequently confirmed by Sullivan.[6] Leicester,[7] we, and perhaps others, have wondered whether the enzyme tyrosinase is necessary for the browning of the human carious lesion. The following 3 studies were devised to evaluate this hypothesis.

TYROSINASE CONTENT OF HUMAN WHOLE SALIVA; TYROSINASE FORMATION BY PURE CULTURES OF ORAL MICROORGANISMS ASSOCIATED WITH HUMAN DENTAL CARIES

Method.—Tyrosinase activity in human saliva and in broth containing pure cultures of oral microorganisms commonly associated with dental caries was measured by the chronometric method of Miller and Dawson.[8] Determinations were made on 10 samples of from 10 to 25 ml. of whole saliva obtained by paraffin stimulation from arbitrarily selected subjects. The organisms tested for tyrosinase activity were oral strains of lactobacilli, *Streptococcus salivarius, Staphylococcus albus, Candida albicans, Saccharomyces cerevisiae,* and *Actinomyces bovis.* Analyses were performed on 48- and 72-hour thioglycollate-dextrose and yeast extract–dextrose broth cultures of each of these organisms. The sensitivity of the method was checked against uninoculated broth samples containing graduated amounts of pure tyrosinase in quantities ranging from 25 to 100 units tyrosinase per 10 ml. broth.

Observations.—The minimum amount of pure tyrosinase measurable by the method used was 3.75 units per ml. of broth. Neither the saliva samples nor the test organisms contained or produced tyrosinase in amounts detectable by this method.

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DISCOLORATION OF THE PROTEIN MATRIX OF HUMAN TOOTH CROWNS BY ORAL LACTOBACILLI IN THE ABSENCE OF TYROSINASE

Method.—The ability of noncarious extracted human tooth crowns to decalcify and discolor in the absence of tyrosinase was determined by exposing sterile specimens to the action of a pure culture of lactobacilli recovered from the saliva of a patient with active and rampant dental caries. A 24-hour broth culture of this organism was inoculated into tubes containing the sterile human tooth crown, 10 ml. yeast extract–dextrose broth and 0.5 ml. 0.01 M sodium diethyldithiocarbamate. Sodium diethyldithiocarbamate has been shown to inhibit tyrosinase by chelating copper, an integral part of the tyrosinase molecule required for enzymatic activity.[9] The tubes were incubated at 37° C. for 24 hours whereupon the broth was decanted and replaced with an identical quantity of medium and chelating agent. This was repeated each day for 30 consecutive days. The tooth fragments were then tested for melanoidins by the method reported by Deakins.[1]

As a control procedure designed to demonstrate the inactivating effect of sodium diethyldithiocarbamate on tyrosinase and the capacity of tyrosinase to react with coronal proteins to form an insoluble black pigment, 50 units of pure tyrosinase were added to each of four tubes of yeast extract–dextrose broth containing sterile decalcified tooth crowns. Sodium diethyldithiocarbamate was added in an amount of 0.5 ml. 0.01 M solution to two of these tubes. All were incubated at 37° C. for 30 days at the end of which time the tooth sections were analyzed for melanoidins.

Observations.—Gross defects in the continuity of the enamel surface indicative of decalcification were noted on the seventh day of exposure of the tooth crowns to the pure culture of oral lactobacilli. A yellowish color in the involved area was observed on the thirteenth day. The intensity of the decalcification and discoloration increased progressively throughout the remainder of the experimental period despite the presence of the copper chelating agent in the medium. By the thirtieth day the decalcified areas were deep brown in color. The browning followed the course of the dentinal tubules and interred substances. Upon subjecting the specimens to the test for melanoidins, black insoluble pigment granules were recovered from each of the crowns so treated.

The samples of decalcified coronal protein subjected to the action of 50 units tyrosinase assumed a yellow-brown color on the third day of incubation. By the thirtieth day the specimens were coal black and gave a positive test for melanoidins. In contrast, the samples in the broth containing 50 units tyrosinase and 0.5 ml. 0.01 M sodium diethyldithiocarbamate failed to undergo a color change and did not yield any pigment granules when analyzed by the Deakins method.

THE TOTAL CARBONYL COMPOUND CONTENT OF PARAFFIN-STIMULATED HUMAN WHOLE SALIVA AND THE EFFECT OF COMPARABLE CONCENTRATIONS OF DIHYDROXYACETONE AND GLYCERALDEHYDE ON THE BROWNING OF ORGANIC MATRICES OF HUMAN TEETH

Method.—The total carbonyl compound content of paraffin-stimulated human whole saliva was determined in 20 samples obtained from 20 unselected subjects. Each sample contained 20 ml. and was collected between 8:00 and 10:00 A.M. The analyses were made by the colorimetric method developed by Friedemann and described by Kinney.[10] A Coleman Model 14 Universal spectrophotometer set at a wave length of 420 mμ was used for this purpose. The concentration in each sample was calculated in terms of milligrams per 100 ml. of saliva.

Sterile noncarious human tooth crowns decalcified for 24 hours in 5 per cent HCl were placed in tubes containing 10 ml. yeast extract–dextrose broth and either dihydroxyacetone or dl glyceraldehyde in amounts equal by weight to the mean concentration of total carbonyl compounds in the 20 samples. These tubes together with similarly prepared control tubes lacking in added dihydroxyacetone and dl glyceraldehyde were incubated at 37° C. The broth in each tube was decanted every 24 hours and replaced with an equal amount of identical medium. This procedure was repeated for 30 days at the end of which time the tooth specimens were tested for melanoidins.

Observations.—The total carbonyl compound levels of the 20 samples ranged from 2.2 mg. per 100 ml. to 27 mg. per 100 ml. with a mean of 15.3 mg. per 100 ml. The decalcified tooth crowns in the tubes containing 15.3 mg. of added dihydroxyacetone or dl glyceraldehyde underwent a color change beginning on the fifth day which progressed from yellow to amber to deep brown by the end of the 30-day experimental period. No color changes were noted in the control teeth. Black insoluble pigment granules were recovered from the sections subjected to the action of dihydroxyacetone and dl glyceraldehyde whereas the control teeth were free of insoluble pigment.

DISCUSSION

The amino acids, tyrosine and phenylalanine, are the usual precursors of melanin in biologic systems. Each has been found in the organic fraction of human teeth.[11] In the presence of tyrosinase and molecular oxygen they are oxidized sequentially to dopa and to dopa quinone. Further stages in the conversion to melanin may proceed in the absence of the tyrosinase although the rate is greatly increased by its presence.[12] The optimum pH for tyrosinase activity is between 6.7 and 7.2. The present studies show that whereas the organic matrix of human teeth forms a black insoluble pigment in the presence of tyrosinase, this enzyme is not present in human saliva in detectable amounts nor is it produced in measurable quantities by test strains of oral microorganisms implicated in the caries process. The findings do not exclude the possibility that tyrosinase may be introduced into the mouth by ingested foodstuffs or by tyrosinase-forming transient members of the oral flora. Whether these sources of tyrosinase are sufficient to induce browning in carious teeth is questionable.

In a review of the literature relative to reactions between sugars and proteins, Danehy and Pigman[13] reported that the evidence is convincing that combinations occur between the amino groups of proteins or amino acids and the aldehyde groups of sugars and their degradation products. They note that the initial stages of condensation are followed by melanoidin formation. The data reported herein indicate that melanoidin formation occurs readily between carbonyl containing degradation products of glucose (dihydroxyacetone and dl glyceraldehyde) and the organic fraction of tooth structure. The demonstration that this reaction occurs at levels of carbonyl compounds comparable to those of saliva supports the hypothesis that conditions favorable to the browning of carious lesions in the absence of tyrosinase exist in the oral cavity.

SUMMARY AND CONCLUSIONS

This study extends previous observations concerning browning of the experimentally induced carious lesion. The browning was produced in the absence of tyrosinase by exposing the organic matrices of human teeth to dihydroxyacetone and dl glyceraldehyde, degradation products of glucose.

The findings support the concept that the melanin-like pigment formed in human dental caries results from the interaction between carbohydrates and proteins.

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THE EFFECTIVENESS OF A TYROTHRICIN DENTIFRICE IN THE CONTROL OF DENTAL CARIES

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TOOTHBRUSHING with a dentifrice immediately after ingestion of food has been reported to be effective in reducing dental caries.[1] There are questions as to whether this means of controlling dental caries is generally practicable, since a family dental survey[2] has shown that only about 30 per cent of the people brush their teeth immediately after each meal. It would seem to be desirable to develop a dentifrice which would have an inhibitory effect on dental caries when the dentifrice is used in the manner that is customary for the majority of people. This goal might be achieved by the addition of agents proposed for the control of caries[3] to a conventional cleansing dentifrice.

An in vitro screening program was applied to a large group of compounds.[4] The results seemed to favor tyrothricin as an inhibitory agent. In contrast to this finding is the published literature on tyrothricin, which is nearly all unfavorable. Negative results were obtained as to its absorption on dental plaques,[5] its effectiveness in dentifrices for reducing lactobacillus counts in human saliva,[6] and its influence in toothbrushing experiments on experimental caries in hamsters.[7] It seemed possible that these negative results in dentifrice studies could be due to the absorption or the precipitation of the tyrothricin by the polishing agent used in the dentifrice, resulting in a loss of effectiveness of the tyrothricin. It was suggested that activity of tyrothricin is retained when a component of the polishing agent in tooth paste is insoluble sodium metaphosphate, and that the degree of retention of activity might depend upon the composition of the dentifrice.

The purpose of this study was to evaluate the effectiveness of toothbrushing with a tyrothricin dentifrice in the control of dental caries in hamsters; to select a dentifrice containing tyrothricin and insoluble sodium metaphosphate for a clinical study with children; and to evaluate the capacity of this dentifrice for clinical control of human dental caries.

EXPERIMENTAL CARIES IN HAMSTERS

Six dentifrices, 1 control and 5 containing tyrothricin, were evaluated for their effect in reducing hamster caries, as a preliminary to a study of their effect on the incidence of human caries. The dentifrices were all in paste

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form, and were coded. The content of each dentifrice was unknown to the investigator until after the hamster jaws had been scored. The essential features of the six dentifrices are shown in Table I.

TABLE I

CODE NUMBER	POLISHING AGENT	TYROTHRICIN (PER CENT)
24	IMP-DCP	0.00*
35	IMP-DCP	0.01
25	IMP-DCP	0.025
65	IMP-DCP	0.05†
15	IMP-DCP	0.05†
74	DCP	0.05

IMP = insoluble sodium metaphosphate.
DCP = dicalcium phosphate.
* = Control.
†Nos. 15 and 65 differ only in the nature of the gum used. Except for a slight change in flavor, No. 15 is the dentifrice used in the clinical study.

Two hundred and forty Syrian hamsters, 3 to 4 weeks old, were divided into 6 groups. Three-fourths were litter mates and were distributed to provide litter mate controls. An equal number of males and females was present in each group. The animals were maintained 5 to a cage.

Six animals were sacrificed at the beginning of the experiment to check for caries. None was present.

The animals were fed a caries-producing diet consisting of: fine cornstarch, 55 per cent; powdered milk, 25 per cent; powdered sugar, 15 per cent; alfalfa, 4 per cent; and NaCl, 1 per cent.

The diet and water were available ad libitum. Carrots were added twice weekly. The groups of animals were numbered to correspond with the number of the dentifrice with which the teeth of each group were brushed. The teeth were brushed daily, by means of a mascara brush whose bristles had been trimmed to a length of 3 or 4 mm. A separate brush was used for each experimental group. The loose tissue of the back of the neck of an animal was pulled back, forcing the animal to open its mouth, exposing the teeth for brushing.

Six animals were sacrificed after 60 days and examined for caries. Since caries was relatively slight at this time, the experiment was continued for 90 days. At this time, the animals were sacrificed and the jaws prepared for examination. The presence of caries was determined by examining the jaws under a dissecting microscope (9×) with a fine probe. The caries was charted on standard forms. A total of 135 animals were available for evaluation at the end of the experiment. The remaining animals were lost by cannibalism.

The results are shown in Table II. The percentage figures are obtained by comparison with paste No. 24, the control dentifrice. Paste No. 15 (insoluble sodium metaphosphate-dicalcium phosphate polishing agent plus 0.05 per cent tyrothricin) proved to be the most effective, producing a 25.6 per cent reduction in the number of carious surfaces. Paste No. 65 (same as No. 15 except for different gum) was only slightly less effective, producing a reduction of 24.2 per cent in the number of carious surfaces. Pastes No. 25 (0.025

TABLE II

HAMSTER CARIES STUDY RESULTS

GROUP	24	35	25	65	15	74
Number of animals	25	26	20	25	23	16
Total carious surfaces	689	608	453	522	472	504
Mean number of carious surfaces	27.6	23.4	22.6	20.9	20.5	31.5
Standard deviation	3.45	4.83	5.16	6.6	4.9	3.12
Standard error of mean	.704	.966	1.19	1.35	1.05	.806
Difference in means*		4.2	5.0	6.7	7.1	3.9†
Standard error of the difference between means*		1.19	1.38	1.52	1.26	1.06
"t" value*		3.52	3.56	4.38	5.60	3.68
Per cent reduction in carious surfaces		15.2	17.8	24.2	25.6	14.3†

*Compared with control Group 24.
†Increase.

per cent tyrothricin) and No. 35 (0.01 per cent tyrothricin) produced reductions of 17.8 per cent and 15.2 per cent, respectively. The statistical analysis of this data is included in Table II. The "t" values[8] obtained are quite high, demonstrating the results to be highly significant (better than the 1 per cent level). It is noteworthy that the inhibition increased as the concentration of tyrothricin increased. Paste No. 74 contained dicalcium phosphate as polishing agent with 0.05 per cent added tyrothricin; this produced a 14.3 per cent increase in the number of carious surfaces which is also statistically significant. There is no explanation for the increased caries activity in Group 74.

CLINICAL CARIES STUDY

This study was carried out in the public school system of a Massachusetts residential community with a population of 7,500. Children 7 to 14 years of age were selected because of their high caries susceptibility which should provide the most severe test of the efficacy of an agent in reducing the incidence of caries. Furthermore, by classroom instruction it is relatively simple to demonstrate to both the experimental and control group the same oral hygiene procedures in order to maintain identical conditions except for the one factor to be varied, the presence of tyrothricin in one dentifrice.

A toothbrushing program was set up as part of a school-wide dental health program, supervised by a dentist and a dental hygienist. At the beginning, full mouth radiographic and clinical examinations were made of each participant. All examinations were made by the same dentist by mirror and explorer, in a dental chair with good illumination. All radiographs were mounted for accurate interpretation. After the initial examinations were completed, a total of 425 children 7 to 13 years of age, were divided into 2 groups of similar age and sex distribution. Insofar as possible, each classroom belonged to the same experimental group. One group used dentifrice No. 15; the other dentifrice No. 24. Dentifrices No. 15 and No. 24 were identical, except that No. 15 contained 0.05 per cent U.S.P. tyrothricin. The polishing agent was a combination of insoluble sodium metaphosphate and dicalcium phosphate. The dentifrices were distributed for school and home use following the completion of the examinations and grouping. Table III shows the

mean number of permanent teeth present in each group at the start of the experiment and at the end of the first year; the similarity of the 2 groups and the lack of significant difference are shown. During the school year, the children brushed their teeth daily during class, just after the luncheon recess. Each child was provided with fresh paper napkins, paper cups, and water for the brushing exercise. Throughout the school year, the dentist and hygienist assisted in the supervision of the classroom brushing, and gave instruction and encouragement in good dental hygiene. Throughout the course of the experiment the experimental and control groups were treated alike. Both groups were given exactly the same instructions in oral hygiene procedures. A certain amount of operative dentistry was performed for the participants by the dentist. The dental hygienist kept a record of the code of the dentifrice given to each child to be certain that each one continued to use only one dentifrice. During school vacations, including that of the summer, the children were given adequate supplies of dentifrice to last until school reopened.

At 1 and at 2 years following the start of the study, the clinical and radiographic examinations were repeated under the same conditions just described, and by the same dentist. The results of the previous examinations were not available to the examining dentist at the time of the subsequent examinations. After all the re-examinations were completed, the records of each first and second examination and first and third were compared and the increments in dental caries noted. No evaluation was made of the progress of caries in deciduous teeth.

TABLE III

COMPARISON OF MEAN NUMBER OF PERMANENT TEETH IN FIRST YEAR CLINICAL CARIES STUDY

AGE AT SECOND EXAM.	FIRST EXAMINATION				SECOND EXAMINATION			
	EXPERIMENTAL (NO. 15)		CONTROL (NO. 24)		EXPERIMENTAL (NO. 15)		CONTROL (NO. 24)	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
8	7.38	6.77	7.38	7.67	11.12	11.69	11.31	10.78
9	11.06	10.18	10.35	11.86	14.47	14.91	13.04	15.57
10	10.87	11.36	11.69	13.60	16.50	16.18	17.12	18.65
11	12.69	15.83	16.30	18.33	18.92	23.33	21.00	24.42
12	17.13	19.54	17.84	19.93	23.53	23.92	23.79	24.87
13	22.67	25.41	24.00	24.56	26.53	26.71	27.50	26.91
Mean	14.46		15.39		19.17		19.57	
S.D.	6.67		6.65		6.36		6.69	
D.F.	350				350			
F	1.006 ("t" ratio valid)				1.103 ("t" ratio valid)			
"t"	1.328 (not significant)				0.58 (not significant)			

Table III shows that the experimental and control groups are sufficiently similar at the beginning and end of the first year of the study, from the standpoint of the mean numbers of permanent teeth. This condition is essential in order to assume that any variation in results will be due to variations in experimental factors. While the mean number of permanent teeth in both groups increased during the year, the individual means closely parallel one another and again show no significant difference. This is most important, since the increment in dental caries depends at least in part on the number of teeth present in the mouth.

The number of new decayed and filled surfaces that occurred during the study on the surfaces that were sound at the beginning of the study were compared. Surfaces that were involved at the beginning were not utilized in computing results, since it is difficult or impossible to know whether or how much they are altered by dental treatment during the course of the experiment. New decayed and filled surfaces were considered as an entity, since they are not altered by dental treatment.

Table IV shows the incidence of new decayed and filled surfaces of all age groups according to sex. Each age group was analyzed statistically to determine whether or not there was a significant difference in the caries experience of males and females of a given age group within each experimental group. If males and females have the same incidence of caries in this study, the data could be pooled without regard to sex. The ratio of the variances[8] (F test) was calculated to determine the validity of the use of the "t" ratio. In the 13-year-olds of Group 15 and the 11- and 13-year-olds of the control group (dentifrice No. 24), the ratio of the variances exceeds that permissible in data to be compared by the "t" ratio. Therefore, the comparisons are made by the method of Cochran and Cox.[8] By this method, however, only the incidence of new caries in the 11-year-olds of the control group (No. 24) showed a significant difference between sexes.

TABLE IV
MEAN NUMBER OF NEW DECAYED AND FILLED SURFACES PER CHILD FOR THE FIRST YEAR OF THE STUDY

AGE AT SECOND EXAM.		EXPERIMENTAL (NO. 15)				CONTROL (NO. 24)				CRITICAL RATIO NO. 15 VS. NO. 24	PER CENT REDUCTION
		N	MEAN	S.D.	CRITICAL RATIO M VS. F	N	MEAN	S.D.	CRITICAL RATIO M VS. F		
8	M	8	5.00	3.32	0.639	16	4.75	3.01	0.227		
	F	13	4.15	2.45		9	4.44	3.68			
	M + F	21	4.48	2.83		25	4.64	3.28		0.163	3.4
9	M	17	2.94	2.71	0.097	26	5.23	3.56	0.941		
	F	22	2.86	2.31		14	6.36	5.22			
	M + F	39	2.90	2.49		40	5.62	4.25		r_1 3.43*	48.4*
										r_2 2.61	
10	M	8	3.25	2.63	0.183	16	4.69	3.11	0.640		
	F	11	3.54	3.61		20	4.85	3.17			
	M + F	19	3.42	3.23		36	4.78	3.15		1.47	28.4
11	M	13	2.31	2.61	1.615	9	2.78	2.61	r_1 3.31*		
	F	12	3.83	1.82		12	9.67	6.10	r_2 3.19		
	M + F	25	3.04	2.41		21	M-2.78	2.61		r_1 2.56*	M = 16.9
							F-9.67	6.10		r_2 2.52	F = 60.3*
12	M	15	3.53	3.86	0.045	19	8.05	6.79	0.071		
	F	13	3.46	3.94		15	8.33	6.73			
	M + F	28	3.50	3.89		34	8.18	6.76		r_1 3.36*	
										r_2 2.46	57.2*
13	M	15	9.13	7.04	r_1 1.23	12	12.67	10.10	r_1 1.78		
	F	14	6.50	3.62	r_2 2.13	23	7.04	4.36	r_2 2.19		
	M + F	29	7.86	5.81		35	8.97	7.38		0.653	12.4
Mean		161	4.19	4.07		191	6.52	4.64			35*

F = 1,297 ("t" ratio is valid).
"t" = 5.04.
DF = 350.
Probability—less than 0.01.
*Significant at the 1 per cent level.

Table IV also shows the mean number of new decayed and filled surfaces obtained by combining males and females of each age group except when not indicated due to a significant difference in the new caries experience of males and females. F tests were calculated again to check on the validity of the "t" ratio in comparing means. The 9-, 11-, and 12-year-olds' ratio of variance indicated the need for the Cochran and Cox method instead of the "t" ratio. Tests of significance are given for the differences between the means of the experimental and control groups for each age. Statistical significance, when present, is noted by an asterisk. The "t" ratio is used unless otherwise noted by "r," which indicates the ratio of Cochran and Cox. The experimental group (No. 15) consistently shows less new caries experience than the control group (No. 24). This reduction ranges from 3.4 per cent to 60.3 per cent. The smallest difference is seen between the 8-year-old groups, presumably because of the teeth which are present among these children. Since this group was 7 years of age at the beginning of this study, only the first permanent molars and the incisor teeth (in varying numbers) are present. Of these teeth, only the first molars are caries susceptible at this age. These teeth become carious in most instances. This extreme in caries attack rate, as well as its localization, may mask or nullify the effect of caries-inhibiting agents in this age group. The greatest reduction in caries experience afforded by dentifrice No. 15 is seen in the 9-, 11-, and 12-year-old groups, which showed 48.4 per cent, 60.3 per cent (males only) and 57.2 per cent reductions, respectively. These changes are found to be significant at the 1 per cent level.

Considering each group as a whole, those children using dentifrice No. 15 averaged 4.19 new decayed and filled surfaces per child; those using dentifrice No. 24 averaged 6.52 new decayed and filled surfaces per child. On the average, those children using dentifrice No. 15 experienced 35.7 per cent fewer new decayed and filled surfaces than the children using dentifrice No. 24. In Table IV there is a statistical analysis of the pooled data. The difference between the means of the group is significant at the 1 per cent level.

The results of the study over a 2-year period are shown in Tables V and VI. The numbers of permanent teeth present at the times of the first and second examinations have been recalculated for those 284 children who were available for re-examination after the 2-year brushing period. Table V shows the mean number of erupted permanent teeth among 264 of these 284 children, initially (first examination) and 2 years later (third examination). At the beginning and end of the 2-year experimental period, both the experimental and control group had the same number of erupted permanent teeth available to become carious.

Table VI shows the mean number of new decayed and filled surfaces that resulted during the 2-year period. A "t" ratio was employed for comparing the means. In order to check the validity of these "t" ratios, Snedecor's F ratio was calculated. In the 12-, 13-, and 14-year-olds, the ratio of variance indicated the need for the Cochran and Cox method instead of the "t" ratio. A significant value (5 per cent level) was found for the 10-year-olds. Although

TABLE V

COMPARISON OF MEAN NUMBER OF PERMANENT TEETH IN TWO-YEAR CLINICAL CARIES STUDY

AGE AT THIRD EXAM.	FIRST EXAMINATION				THIRD EXAMINATION			
	EXPERIMENTAL (NO. 15)		CONTROL (NO. 24)		EXPERIMENTAL (NO. 15)		CONTROL (NO. 24)	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
9	6.88	7.08	7.20	7.89	12.25	15.31	13.80	14.67
10	11.25	10.36	10.00	11.71	18.75	19.09	15.25	19.72
11	10.88	13.91	11.60	13.40	19.62	21.64	21.40	22.35
12	15.25	16.33	16.12	18.27	24.92	26.00	24.12	26.18
13	17.87	19.25	18.78	20.71	26.27	26.62	24.94	27.00
14+	Insufficient subjects for detailed analysis				Insufficient subjects for detailed analysis			
Mean	14		14		21		21	
S.D.	6.12		5.80		5.88		6.05	
D.F.	282				282			
F	1.12 ("t" test is valid)				1.05 ("t" ratio valid)			
"t"	0.32 (not significant)				0.59 (not significant)			

TABLE VI

MEAN NUMBER OF NEW DECAYED AND FILLED SURFACES PER CHILD FOR THE TWO-YEAR STUDY

AGE AT THIRD EXAM.	EXPERIMENTAL (NO. 15)				CONTROL (NO. 24)				CRITICAL RATIO NO. 15 VS. NO. 24	PER CENT REDUCTION
	N	MEAN	S.D.	CRITICAL RATIO M VS. F	N	MEAN	S.D.	CRITICAL RATIO M VS. F		
9 M	8	9.9	4.12	1.53	15	7.2	4.22	0.97		
9 F	13	6.9	4.12		9	9.6	7.10			
9 M + F	21	8.0	4.36		24	8.1	5.60		0.02	1.2
10 M	16	5.9	5.16	0.38	19	8.5	4.63	0.90		
10 F	22	6.5	4.88		14	10.0	4.94			
10 M + F	38	6.2	5.00		33	9.1	4.82		2.36 (5%)	31.8
11 M	8	4.4	2.28	1.83	15	6.7	4.53	0.75		
11 F	11	8.4	5.57		20	8.2	6.16			
11 M + F	19	6.7	4.91		35	7.6	5.56		0.58	11.8
12 M	12	9.4	6.84	0.50	8	9.0	11.14	1.47		
12 F	12	8.1	5.79		11	16.6	9.84			
12 M + F	24	8.8	6.37		19	13.4	11.07		1.74	34.3
13 M	15	8.8	6.51	0.25	18	13.5	10.51	0.12		
13 F	8	9.5	5.37		14	13.1	9.04			
13 M + F	23	9.0	6.15		32	13.3	9.90		1.86	32.3
14 M—Insufficient subjects for detailed analysis					Insufficient subjects for detailed analysis					
14 F—										
14 M + F	9	9.1	7.66		7	18.1	11.07		1.82	49.7
Mean	134	7.7	5.71		150	10.4	8.27		2.58	26*

F = 2.10.
"t" = 2.59.
DF = 282
*Significant at the 1 per cent level.

the 12-, 13-, and 14-year-old groups were not significant, their ratios were border line. The percentage reductions in the incidence of new decayed and filled surfaces vary from 49 per cent in the case of the 14-year-olds to 32 per cent, 34 per cent, and 32 per cent, respectively, in the 10- 12-, and 13-year-olds. As noted in the first year's results, the least effect was noted in the youngest age group. The pooled data of all age groups are included in Table VI. The

experimental group had a mean of 7.7, and the control a mean of 10.4 new decayed and filled surfaces during the 2-year brushing period. Brushing with the dentifrice containing 0.05 per cent tyrothricin resulted in about 26 per cent reduction in the incidence of new decayed and filled surfaces. This figure is significant at the 1 per cent level.

SUMMARY

A group of tyrothricin tooth pastes containing insoluble sodium metaphosphate as a component of the polishing agent produced a reduction in hamster caries in comparison with a control tooth paste.

A selected tooth paste containing 0.05 per cent tyrothricin and insoluble sodium metaphosphate as a component of the polishing agent was found effective in reducing dental caries in children in a controlled clinical study. The incidence of new decayed and filled permanent tooth surfaces was reduced by 35 per cent after 1 year and by 26 per cent after a total of 2 years' brushing with the tyrothricin tooth paste.

The reductions in caries are highly significant, and may be ascribed to the presence of the tyrothricin, since identical oral hygiene procedures were followed in both experimental and control groups.

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A TECHNIC FOR THE RADIOGRAPHIC VISUALIZATION OF THE VASCULAR SYSTEM OF THE PERIODONTAL TISSUES*

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THE intravascular injection of such materials as dye solutions, radiopaque mass, and plastic has been of marked value in the study of vascular pathways, both normal and pathologic. This tool has not been utilized to any great extent for the study of vascular changes in the dental apparatus. It is felt that there is a need for the development of an injection technic which incorporates a radiopaque mass so that vascular supply could be easily visualized on x-ray film. This technic has been developed for the study of the vascularity of the gingivae using a mercury-gelatin mass.

In the past, uses of injection technics have fallen into 3 major categories: (1) tracing vascular anatomy; (2) studying collateral circulation around infarcted areas; and (3) tracing the possible hematogenous dissemination of malignant tumors.

Horton[1] used metallic mercury to inject the arteries of 42 amputated extremities and dissected them using the radiographs as guides. This study was a correlation of collateral circulation with extent of grangrene. Schlesinger[2] reported a technic using injections of radiopaque mass to study the coronary arteries and the relationship of vascular supply to areas of myocardial infarction. Batson[3] injected both artist's tube water color and colloidal thorium dioxide into the deep dorsal veins of the penis in both human cadavers and monkeys and found that there was a pathway between the pelvic organs and the vertebral plexus of veins. Liebow, Hales, Lindskog, and Bloomer[4] have developed a technic for the injection of colored vinylite into the circulatory pathways of the lungs and then dissolving away the parenchyma. This method produces a plastic model of the circulatory tree. Anderson[5] also showed by injection of Diodrast into the deep dorsal veins of the penis of cadavers that pelvic neoplasms probably metastasize through the vertebral and cranial venous systems to the brain.

Keller and Cohen[6] have recently reported the use of India ink perfusions to study the vascular plexus of the oral tissues. In this technic it is necessary to control the particle size of the mass. They perfected a method of cutting the hard tissues involved and of clearing the specimens for mounting.

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METHOD

In this study, a mercury-gelatin mass (developed by Dr. Leo Reiner of the Beth Israel Hospital, Boston) was used which met the following basic requirements: (1) it should not be injurious to the tissues; (2) it should have sufficient radiopacity to allow visualization of small vessels, particularly when they cross bone and tooth; (3) it should remain within the vascular tree; (4) it should allow for a fairly rapid injection; (5) it should be possible to harden the mass after injection; (6) it should contain no large particles which might produce artifactual occlusions; (7) it should be readily removable during the dissection of the vessels; (8) it should not interfere with preparation of microscopic sections if left in the specimen; and (9) the size of the vessels to which the mass flows should be known. We found that a mercury and 10 per cent gelatin mass in proper proportions adequately met the above specifications.

Careful preparation of the mass is important because small variations may adversely affect the injection.

Ten Per Cent Gelatin Mixture.—

1. In a 1,000 c.c. beaker, place 400 c.c. of 2.5 M KI.
2. Add 2.5 c.c. liquid phenol (liquified phenol U. S. P.).
3. Add 100 Gm. of Difco Bacto gelatin very slowly through a small funnel. Regulate the automatic stirrer to that speed which will best facilitate the dissolving of the gelatin. Stir until all the gelatin is dissolved. A few drops of caprylic alcohol may be added to control frothing if necessary.
4. Add 10 c.c. of 3 M NaH_2PO_4.
5. Add 500 c.c. H_2O and mix well.
6. Store the 10 per cent gelatin mixture in a glass bottle that is well stoppered.

3M NaH_2PO_4 (sodium acid phosphate).—

1. In a 2,000 c.c. Erlenmeyer flask place 414 Gm. of NaH_2PO_4 H_2O (CP).
2. Add 813 c.c. of distilled H_2O.
3. Dissolve over heat.
4. Filter through a Buchner funnel.
5. Store in a well-stoppered glass bottle.

2.5 M KI.—

1. In a 2,000 c.c. Erlenmeyer flask place 890 c.c. of distilled H_2O.
2. Add 415 Gm. of potassium iodide crystals (U.S.P.).
3. When dissolved, filter through a Buchner funnel.
4. Store and let reach room temperature in a well-stoppered glass bottle.

The mercury used was a standard brand of the triple-distilled metal. A few minutes prior to injection, 60 c.c. of the 10 per cent gelatin mixture and 60 c.c. of the triple-distilled mercury were mixed for 2 minutes in a Waring Blender.

Keller and Cohen[6] utilized a modified pump which gave a pulsating type of injection. Schlesinger, on the other hand, felt that this was uneecessary. It was further shown by Wessler and Schlesinger[8] that a constant injection

pressure of 400 mm. Hg did not result in intimal tears, vascular rupture, or extravasation. We have successfully adopted a constant pressure of 400 mm. Hg controlled from a compressed air source.

PROCEDURE

The dog was anesthetized with intravenous injection of 4 per cent Nembutal solution (dosage: 2.5 c.c. per 10 pounds of body weight). A midline incision was made in the neck. On one side the common carotid artery and jugular veins were dissected out and isolated with gauze. Two cubic centimeters of heparin was then injected into the common carotid artery. The injection reservoir was filled with normal saline solution to which had been added 2 c.c. of heparin. All tubing was cleared of any air. The common carotid artery was catheterized, the jugular vein cut, and the animal perfused with 1 L. of normal saline solution. The reservoir was filled with the mercury-gelatin mass,

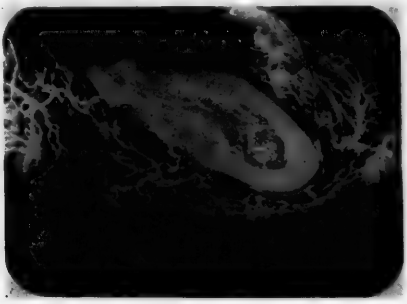

Fig. 1.—A representative radiograph produced by the perfusion technic of a radiopaque mass.

the air cleared from the tubing, and the common carotid artery ligated proximal to the cannula. The mass was injected at a constant pressure of 400 mm. Hg and about 100 c.c. was used. At this stage, mercury-gelatin mass was flowing freely from the cut jugular vein. All major neck vessels were then ligated and the animal decapitated. The mandible was propped open. The mass was set by placing the entire head in a crock of formaldehyde for 5 days. The mandible was dissected out and the teeth to be radiographed and studied were cut out in block with an electric saw. Buccolingual radiographs were taken of the blocks thus produced.

FINDINGS

Examination of the radiographs produced by this procedure reveals that the findings of Keller and Cohen[6] are correct in regard to the major vascular supply of the gingivae of the dog. Blood vessels paralleling the periosteum of the mandible are visible in the radiographs (Fig. 1). One finds these vessels in edentulous areas clearly, both buccally and lingually; they anastomose on the crest of the ridge to form a gingival plexus.

SUMMARY

A method has been presented for the radiographic visualization of the vascular system of the jaws in the dog. This tool may be utilized for study of gingival vascular changes in pathologic conditions.

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THE BEHAVIOR OF TRANSSEPTAL FIBERS IN PERIODONTAL DISEASE*

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THE transseptal fibers are those groups of collagen fibers comprising a ligament-like band, arising in the cementum of the proximal surface of a tooth subjacent to the gingival fiber group and running across and above the bone crest to an adjacent tooth. It is seen recurring in each interproximal space attaching one tooth to another and forming the supporting framework for the interproximal gingiva. These fibers, together with the gingival fibers, occupy the crescent-shaped cemental area formed by the convexity of the cemento-enamel junction on the proximal surface.

Although there have been many observations reported concerning the effect of inflammation on the gingival tissues and the alveolar crest, little mention has been made regarding the behavior of the transseptal fibers under this condition. In order to investigate this problem more thoroughly, the following study was undertaken.

MATERIAL AND METHODS

Histologic preparations of human jaw sections stained with hematoxylin and eosin and Wilder's silver stain in a mesiodistal plane illustrating the following situations were studied: (1) when the epithelial attachment is at the cemento-enamel junction; (2) when the epithelial attachment is on the cementum; (3) in instances where the epithelial attachment proliferated apically to the alveolar crest; (4) the interradicular area of maxillary and mandibular molars when the interradicular tissue was partially destroyed; and (5) in edentulous areas between teeth.

FINDINGS

In order to present the essential findings of this study, typical photomicrographs were prepared and are presented.

Fig. 1 is a mesiodistal section of a maxillary second incisor-canine region in which, clinically, a gingivitis was present. Histologically, a gingival inflammation is apparent. The transseptal fiber group can be observed running from one tooth to the other (*T*) in their normal position. The epithelial attachment of both teeth is located at the cemento-enamel junction.

In Fig. 2 the bone crest has been resorbed. The epithelial attachment is located at the cemento-enamel junction and the entire gingival tissue is infiltrated by inflammatory cells, the gingival fibers being almost completely lost.

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Fig. 1.—Photomicrograph of a mesiodistal section of a maxillary second incisor-canine region. Transseptal fibers may be seen at *T*.

Fig. 2.—A high-power photomicrograph disclosing disruption of the gingival fibers. Inflammatory cells can be seen in between the bundles of the transeptal fibers. At *P* a group of fibers originally attached to the alveolar bone may be seen unattached. (*C*, cementum; *P*, periodontal membrane fibers; *B*, bone.)

Fig. 3.—Low-power photomicrograph of a mesiodistal section in advanced marginal periodontitis. (Transeptal fibers may be seen at *T*; at *A* a transeptal fiber group being formed is shown.)

The transseptal fiber group is infiltrated by inflammatory cells. At *P* a group of fibers originally of the periodontal membrane can be seen unattached to the bone.

Fig. 3 are a low- and high-power photomicrographs showing a mesiodistal section of an advanced marginal periodontitis involving 2 maxillary premolars. The interdental crestal bone has been resorbed. At present there is no activity; some recent apposition of bone to the interdental septum can be seen as indicated by the appositional lines at the present crest. Occlusally to the crest are a group of collagen fibers running from one tooth to another (*T*). Between this group of fibers and the crestal bone one can observe another series of fibers which seemingly are not connected at two places (arrows). The high-power

Fig. 4.—Photomicrograph showing several sets of transseptal fibers.

Fig. 5.—High-power photomicrograph illustrating the 2 cemental tears (*CT*). Fibers (*F*) arising from the periodontal membrane (*PM*) attached to the cemental tear can be seen joining (*J*) fibers which are running occlusally to the crestal bone (*C*) attached to the cementum of the other tooth.

photomicrograph illustrates this latter group of fibers. One can surmise that these are the original fibers of the periodontal membrane but are now unattached on the bone side because of the loss of that tissue. In the mid-section of this group are fibers arranged similarly to transseptal fibers which necessarily must be a new formation since no such structures occur normally in this area when the alveolar septum is intact (*A*). One can interpret that the periodontal membrane fibers attached to both teeth are being joined by a new group of collagen fibers.

Occasionally, several sets of fiber-groups can be seen running interdentally from one tooth to another. Such an instance is presented in Fig. 4. In this case the teeth are close together; the interproximal septum being very thin.

Fig. 6.—Low- and high-power photomicrograph in which the epithelial attachment has proliferated alongside the cementum apical to the bone crest. The group of fibers attached to the cementum subjacent to the epithelium pass parallel to the bone, turning to traverse the crest and join fibers to the other tooth. (*EA*, epithelial attachment; *A*, apical proliferation of epithelial attachment.)

Fig. 7.—High- and low-power photomicrograph on an interradicular area of a mandibular molar. The crestal bone has been altered by the disease process and transseptal fibers may be seen extending from one root to the other.

Fig. 5 illustrates the structures between two premolars: (left to right) periodontal membrane, alveolar septum, and periodontal membrane. Apposition of bone onto the septum is seen on the left, while the bone surface on the right is being resorbed. Interpretation of these findings discloses that the teeth are moving to the left. Cemental tears may be seen (*CT*). Of interest is

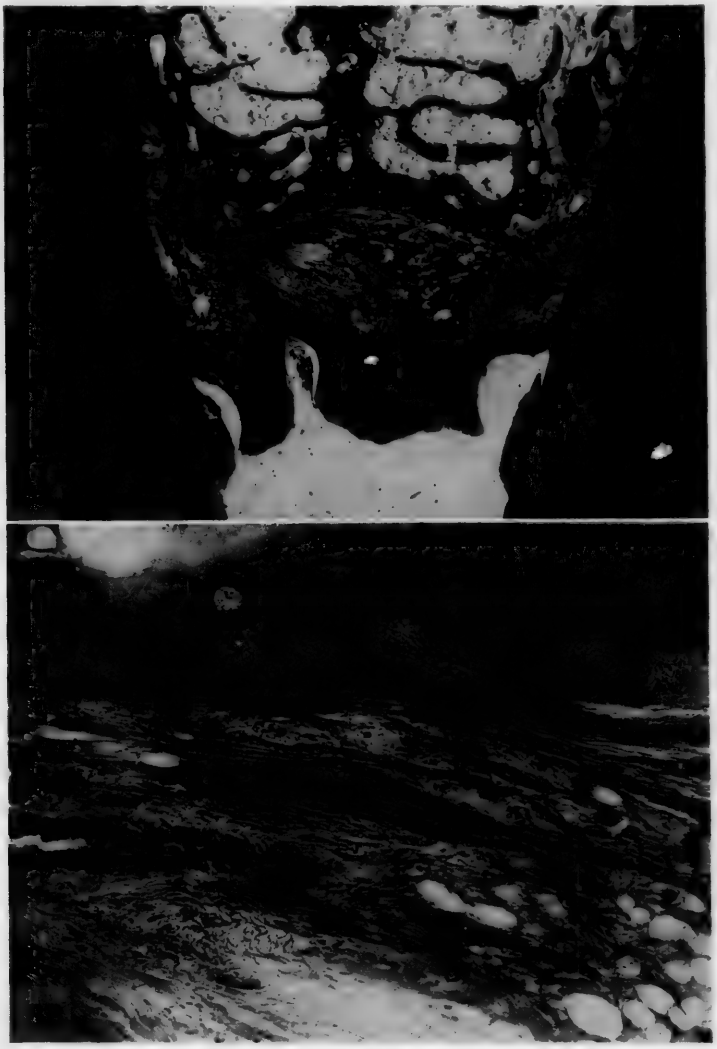

Fig. 8.—High- and low-power photomicrograph of an edentulous area between two teeth. Note that a transseptal group of fibers has formed.

the finding of the original periodontal membrane attached to the cemental tear (*PM*). Fibers connecting to this area can be seen extending occlusally (*F*) and joining (*J*) another group of fibers arising from the cementum of the other tooth which pass occlusally to the bone crest (*C*). These findings substantiate the concept that the new formation of transseptal fibers are a uniting of previously existing periodontal membrane fibers.

Another interesting observation can be made in those instances where the epithelial attachment has migrated apically to the bone crest. In Fig. 6 the epithelial attachment, *EA*, has proliferated alongside the cementum to a point, *A*, on the cemental surface. A group of fibers, attached to the cementum subjacent to the epithelium, run across to the other tooth directly above the bone crest. The high-power photomicrograph illustrates the character of the fiber group between the tooth and the alveolar bone.

Fig. 7 illustrates the interradicular area of a mandibular molar. The crestal bone has been destroyed, the crotch being filled with connective tissue. The collagen fibers have assumed the topography of transseptal fibers. The 2 roots resemble individual teeth in respect to the fiber group. This finding was also observed in the maxillary molar region. In the high-power photomicrograph, one can see that the apical group of the transseptal fibers (arrow) arise from what can be considered periodontal membrane giving added proof that the manner in which the transseptal fibers form is from the union of the original periodontal membrane fibers.

The behavior of the transseptal fibers after the extraction of teeth is similar to the findings once the marginal periodontium is destroyed. In these instances a group of collagen fibers (transseptal group) can be observed bridging the edentulous area, running from one tooth to another. Fig. 8 (low- and high-power photomicrographs) illustrates this bridge between a maxillary first incisor and canine. The arrangement of the collagen fibers into a ligament-like band is apparent; the character of the transseptal fibers can be observed in the high-power photomicrograph.

SUMMARY AND CONCLUSIONS

The transseptal fibers comprise groups of collagen fibers in a ligament-like fashion running from one tooth to another. In lesions in which the marginal periodontium has been destroyed, collagen fibers arranged in similar fashion are seen above the alveolar crest. Occasionally, more than one and, in some instances, several sets may be seen. In instances where the epithelial attachment has migrated apically to the alveolar crest, the fibers arising between the tooth and bone can be seen running alongside the bone and passing over the crest to the adjacent tooth. In edentulous areas between 2 teeth, there is a formation of collagen fibers functioning as a transseptal group. Also, when the interradicular area is exposed, fiber groups can be noted running from one root to another.

There is evidence from histologic study that these groups of fibers are not new formations but are a uniting of previously existing periodontal membrane fibers. This formation can be considered a defense mechanism against destruction of the marginal periodontium due to inflammation in maintaining the contact between teeth and also possible in maintaining stability of the teeth.

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A HISTOCHEMICAL STUDY OF PROTEIN-BOUND SULFHYDRYL AND DISULFIDE GROUPS IN NORMAL AND INFLAMED HUMAN GINGIVA

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CHEMICAL and histochemical studies of epithelium[1, 6] indicate the likelihood that sulfhydryl and disulfide groups are of significance in the metabolism of the gingiva. These substances have been associated with physiologic keratin formation[1, 5] as well as pathologic epithelial changes in certain dermatologic diseases.[1] In addition, it has been shown that these groups are functionally significant in a wide range of biologic activity such as enzymatic and antibody reactions, cell division and growth, cell permeability, detoxification processes and biosynthesis.[7-9]

A histochemical study of the distribution of protein-bound sulfhydryl and disulfide groups in normal and inflamed human gingiva was undertaken for the purpose of exploring the following: (1) hitherto undetected microscopic features in normal and inflamed gingiva; (2) the relationship of the distribution of protein-bound sulfhydryls and disulfides to gingival keratinization; (3) the effect of chronic inflammation upon the distribution of protein-bound sulfhydryls and disulfides.

PROCEDURE

Biopsies of clinically normal and inflamed gingiva were obtained from 35 subjects, 15 males and 20 females, varying in age from 15 to 71 years, and fixed immediately in a 1 per cent solution of trichloroacetic acid in 80 per cent alchohol for 18 hours. From each biopsy 4 slides in serial sections were cut at 7μ and processed as follows: (1) stained with hematoxylin and eosin for purposes of orientation; (2) prepared according to the Barrnett-Seligman[3] histochemical technic to demonstrate both protein-bound sulfhydryls and disulfides; (3) prepared according to the Barrnett-Seligman technic for the demonstration of protein-bound sulfhydryls only[10]; (4) prepared as a control using 0.1 M. n-ethyl maleimide or 0.1 M. iodoacetate to block the sulfhydryl groups, preventing them from reacting with the reagents of the Barrnett-Seligman technic, thus demonstrating the specificity of the latter technic.

With the Barrnett-Seligman technic, a pink or red color indicates the presence of sulfhydryl groups in widely separated molecular arrangement; a bluish color indicates a closer arrangement of sulfhydryl molecules. To detect the presence of disulfides, the tissue is reduced with 0.2 M. thioglycollic acid

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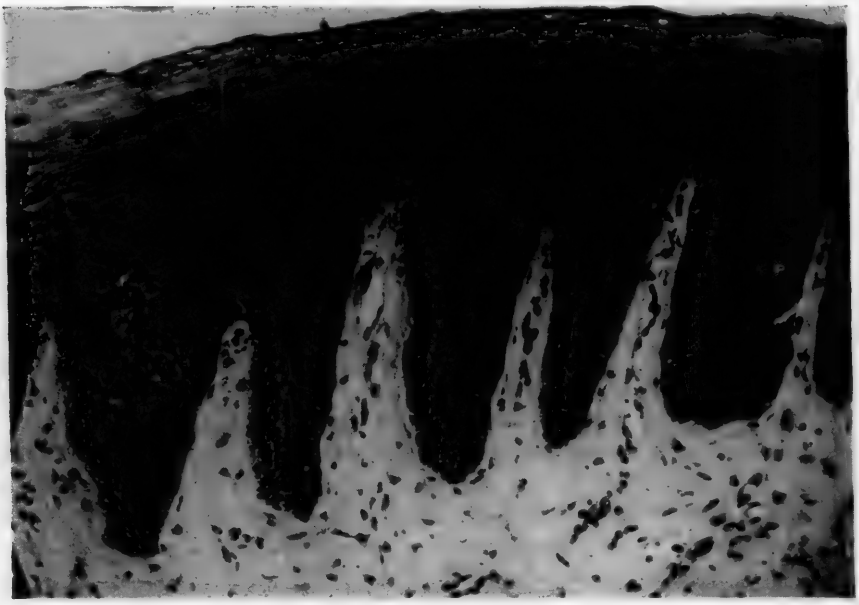

Fig. 1.—Normal gingiva stained with hematoxylin-eosin showing various layers of epitheluim. (Orig. mag. ×140; reduced ¼.)

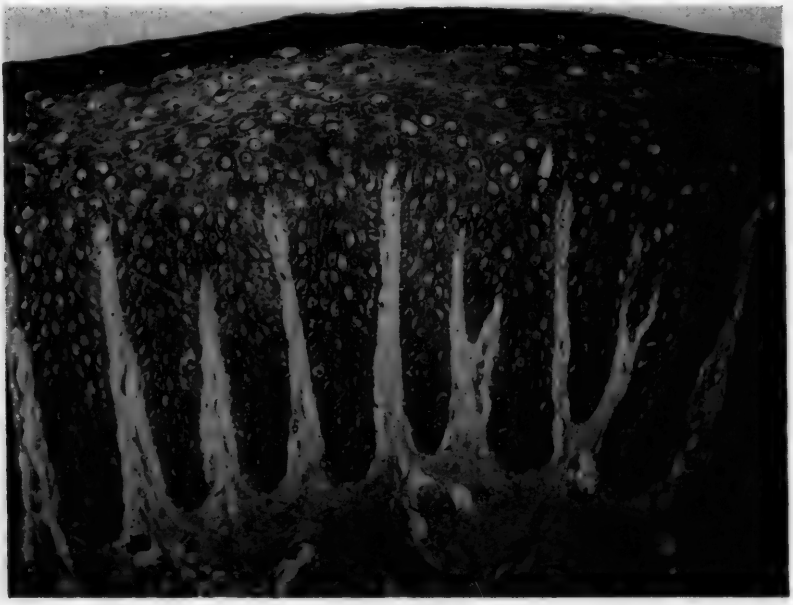

Fig. 2.—Normal gingiva stained with Barrnett-Seligman technic to demonstrate sulfhydryl groups. In the epithelium the malpighian layer stains less intensely than the corneal layer (keratinized and parakeratinized). Sulfhydryls are present in fibroblasts and endothelial cells of connective tissue, and in barely demonstrable amounts in intercellular substance. (Orig. mag. ×140; reduced ¼.)

to convert disulfides to sulfhydryls.[3] An increase in the intensity of sulfhydryl stain following reduction of the tissues is attributed to the pre-existent disulfides.

OBSERVATIONS

Normal Gingiva—Epithelium.—With the Barrnett-Seligman technic the malpighian layer (basal, prickle-cell, and granular) stained less intensely for sulfhydryls than the keratinized and parakeratinized layers (Figs. 1 and 2). In the malpighian layer, protein-bound sulfhydryls were demonstrable in the cytoplasm, the intercellular bridges, and to a lesser degree in the nucleus where they were seen in the chromatin granules and nuclear membrane, and appeared either homogeneous or granular (Fig. 3). At the junction of the epithelium and connective tissue, cytoplasmic processes extended from the basal epithelial cells into the papillary layer of the connective tissue and were reactive for sulfhydryl groups (Fig. 4). In the keratinized and parakeratinized layers the sulfhydryl stain was more intensely red-blue and more homogeneous than the remainder of the epithelium. Keratinized and parakeratinized cells appeared to stain with equal intensity.

Fig. 3.—High-power view of prickle cells showing distribution of protein-bound sulfhydryls in the cytoplasm, in chromatin granules, nuclear membrane, and intercellular bridges. (Barrnett-Seligman technic. Orig. mag. ×950; reduced ¼.)

In sections reduced with thioglycollic acid to localize the disulfide groups, there was an over-all intensification of the sulfhydryl stain throughout the epithelium with no apparent alteration in distribution (Fig. 5).

Normal Gingiva—Connective Tissue.—In the connective tissue, the sulfhydryls were present in minute amounts in the intercellular substance but were prominent in the cytoplasm, cell membrane, and nuclear membrane of the fibroblasts and endothelial cells. The collagen could not be delineated from the ground substance with the Barrnett-Seligman stain.

The distribution of disulfides was the same as that of the sulfhydryls.

Inflamed Gingiva.—The inflammation was chronic in nature with a predominance of plasma cells and lymphocytes in the connective tissue. The

epithelium showed varying degrees of proliferative and degenerative changes. In the proliferating epithelium, the sulfhydryl stain was increased in intensity throughout all the layers except the basal layer. In areas of epithelial degeneration there was an over-all diminution in the intensity of the sulfhydryl stain. The stratum corneum was diminished or absent in areas of pronounced

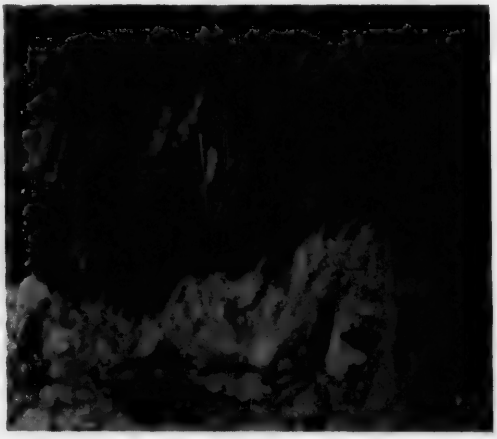

Fig. 4.—High-power view showing sulfhydryl containing cytoplasmic processes extending from basal epithelial cells into underlying connective tissue. (Barrnett-Seligman technic. Orig. mag. ×950; reduced ¼.)

Fig. 5.—Serial section of tissue shown in Fig. 2. This section was treated with thioglycollic acid to reduce disulfides to sulfhydryls prior to staining with the Barrnett-Seligman technic for sulfhydryls. Diffuse intensification of stain in this section (compared with Fig. 2) indicates that disulfides are more or less uniformly distributed throughout epithelium, and are present in small amounts in fibroblasts, endothelial cells, and intercellular substance of connective tissue. (Orig. mag. ×140; reduced ¼.)

epithelial degeneration. In the connective tissue of inflamed gingiva, sulfhydryls were present in the fibrinoid collagen and in the cytoplasm, cell membrane, nuclear membrane, and chromatin granules of the leukocytes and connective tissue cells.

Because of the slight difference between the reduced and nonreduced sections, it was not possible to determine the extent to which disulfides were present in the proliferating epithelium or in the intercellular substance of the connective tissue. Disulfides were detected in the cytoplasm, cell membrane, nuclear membrane and chromatin of the leukocytes and connective tissue cells. The disulfide content of the degenerated epithelium was reduced when compared with the normal.

SUMMARY

Sulfhydryl and disulfide groups were demonstrated in normal and inflamed human gingiva with the Barrnett-Seligman technic. The findings indicate that in normal human gingiva sulfhydryl groups are present in the epithelium and connective tissue. The concentration of sulfhydryls is greater in the keratinized and parakeratinized layers than in the deeper layers of the epithelium. Disulfide groups are distributed uniformly throughout all the epithelial layers. It has been assumed that the process of keratinization entails the oxidation of sulfhydryls to disulfide groups.[1, 5] Our findings show the distribution of both these groups of substances in the gingiva but provide no basis for evaluating the relationships of these substances to the processes of gingival keratinization.

There is an increase in the sulfhydryl content in proliferating epithelium in areas of gingival inflammation. This increase is consistent with the identification of sulfhydryl groups with the processes of cell division and growth.[4, 8] The diminished sulfhydryl and disulfide content in epithelium undergoing degeneration may be attributable to the loss of cellular and intercellular substance caused by the degenerative processes.

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ADJUNCTIVE ASCORBIC ACID ADMINISTRATION IN ANTIBIOTIC THERAPY

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THE enhancement of the serum blood level of antibiotics after oral administration of minimal doses would be highly desirous. In oral administration of the broad spectrum antibiotics, drugs of the tetracycline group such as tetracycline (Achromycin), chlortetracycline (Aureomycin), and oxytetracycline (Terramycin), it has heretofore been laborious to achieve and maintain the desired blood level necessary for effective action. High serum blood levels are maintained only with the use of multiple doses of the chosen drug over an extended period of time. When the antibiotic drugs of the tetracycline groups are administrated in high and frequent doses there is a gradual increase of the blood level of the drug until a point is reached, also varying with the individual, at which the concentration of the drug in the blood does not increase further, even with larger doses of drug. Utilization of high and frequently administered dosages often presents the practitioner with many secondary complications such as nausea, gastrointestinal upset, dermatosis, and increased growth of fungus.

In recent years numerous experimental studies have been reported in the literature concerning the specific adjunctive action of other drugs which contributed to the raising of the blood level of orally administered antibiotics. Paul, Dryer, and Routh[1] found more than a twofold increase in the rate of absorption of salicylates by utilizing aluminum dihydroxyaminoacetate-magnesium carbonate as an adjuvant. This drug acts as a buffer which reduces the deleterious effects on the drug by the gastrointestinal secretions. Utilizing adjuvants such as malonic acid, pyruvic acid, monosodium phosphate, tricarballylic acid and lactic acid, Eisner, Stirn, Dornbush, and Oleson[2] have shown an enhancement of serum concentrations of Aureomycin and Terramycin lasting at least 8 hours in rats. They reported up to a tenfold increase in serum blood levels when the drugs were administered simultaneously with the oral dosages of antibiotics varying from 8 mg. to 200 mg. per kilogram.

This paper presents a preliminary study of a simple method for increasing the serum concentrations of the various tetracycline antibiotics utilizing the adjunctive action of ascorbic acid in oral administration in human beings.

METHOD

This study was conducted on 7 patients. All tests were done on the fasting stomach. Those patients who were given ascorbic acid simultaneously were

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given 250 mg. of antibiotic and 500 mg. of ascorbic acid. Two hours after oral administration of the drugs blood samples were obtained. The same 7 patients were used as controls. In the control tests the antibiotic alone was used. The method used for determining the serum concentration of antibiotic is that described by Schneierson[3] utilizing the procedure based upon the inhibition of a test organism, *Proteus vulgaris OX-19,* by the antibiotic. This prevents the reaction and color change produced in a urea-phenol red medium when the urea is split to ammonia by the test strain.

Achromycin, Aureomycin, and Terramycin were employed in these experiments. Serum determinations were done on the same individual at different times with the use of ascorbic acid, without the ascorbic acid, and varying the antibiotic. Patients may have taken one antibiotic for one test and another the following time. All antibiotic drugs were obtained from a commercial source. The ascorbic acid used was U.S.P. purity. Only single doses were utilized in this study. Seven days elapsed between tests.

RESULTS

Antibiotic Serum Concentration of Single 250 mg. Dose.—

In all the tests conducted after the single dose of antibiotic without the adjuvant, the serum concentration did not rise above 0.5 mcg. per milliliter in any case (Table I). Eisner, Stirn, Dornbush, and Oleson[4] reported that the

TABLE I

ANTIBIOTIC SERUM LEVEL CONCENTRATION WITHOUT AND WITH THE USE OF ASCORBIC ACID ADJUVANT

	DOSE	TIME	DOSE	TIME
PATIENT	250 MG. ANTIBIOTIC*	2 HOUR MCG./ML.	250 MG. ANTIBIOTIC 500 MG. ASCORBIC ACID	2 HOUR MCG./ML.
A.	O-T	0.33	O-T	1.1
A.	T	0.33	T	3.3
B.	O-T	0.33		
B.	C-T	0.25	C-T	3.3
C.	T	0.33		
D.			O-T	1.4
D.			O-T	1.6
E.			C-T	5.0
F.	C-T	0.4	C-T	2.5
F.			T	2.5
G.			T	5.0

*C-T = Chlortetracycline; O-T = Oxytetracycline; T = Tetracycline.

serum concentrations of antibiotics are not directly in proportion to the doses. Their studies on guinea pigs showed that single doses of from 50 mg. to 200 mg. did not give higher serum levels than did the 50 mg. dose. Their single dose experiments with guinea pigs are proportionally similar to our findings in human beings.

Enhancement of Antibiotic Serum Concentration With the Use of Ascorbic Acid Adjuvant.—

The results shown in Table I follow the single dose oral administration of antibiotics (250 mg.) with the simultaneous ingestion of ascorbic acid (500

mg.). Increases of the serum level concentrations of the antibiotic were found to be from 3 to 15 times greater with adjunct ascorbic acid compared to use of the antibiotic alone. At no time was the concentration less than one microgram per milliliter with the utilization of ascorbic acid. Those individuals who had low serum concentrations of antibiotics without the use of the adjunct recorded the highest rise when the ascorbic acid was used.

DISCUSSION

Ascorbic acid appears to be a potent adjunct in rapidly increasing the serum level concentration of antibiotic drugs of the tetracycline group. The enhancement activity by ascorbic acid is substantially the same for tetracycline (Achromycin), oxytetracycline (Terramycin) and chlortetracycline (Aureomycin). This is understandable in view of the close structural similarity of these drugs. This study indicates that ascorbic acid acts as an activator for tetracycline antibiotics making greater amounts available in the blood stream. This has been demonstrated by determinations of the antibiotic serum blood level in human beings after simultaneous oral administration of ascorbic acid and antibiotic drugs. The normal blood levels for a given dose vary with different individuals and in the same individual they may vary from day to day. Thus, a 250 mg. dose of one of the tetracycline drugs alone may result in a blood level of 0.25 mcg. to 1.0 mcg. per milliliter 2 hours after oral administration. When the dose of the tetracycline drug is combined with 500 mg. of ascorbic acid, blood levels 2 hours after administration are found to be increased up to 15 times.

SUMMARY AND CONCLUSION

1. Effectiveness of antibiotic therapy with tetracyclines can be more rapidly induced utilizing minimal doses with the adjunctive use of ascorbic acid.

2. Ascorbic acid produces a rapid increase in serum level concentrations which may eliminate the necessity of frequent and large doses of tetracycline antibiotics for effective therapy.

3. Reduction of prolonged and multiple doses in antibiotic therapy obviates side reactions so often accompanying the use of antibiotics.

4. The effective dose of ascorbic acid was found to be 500 mg. for every 250 mg. of tetracycline antibiotic.

The authors wish to express their appreciation to Dr. S. Stanley Schneierson for his capable technical assistance.

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THE MANDIBULAR SECOND PREMOLARS: MORPHOLOGIC VARIATION AND INHERITANCE

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AT PRESENT, excluding the pathologic syndromes for reasons of their nature and infrequent occurrence, the list of the human traits whose genetic mechanisms are clearly understood is relatively short. Unfortunately for the simplification of any genetic solution to the problems involved, most human traits do not operate by a simple Mendelian method of inheritance. Pleiotropism and penetrance, among other factors, obscure the underlying mechanisms. In such instances the usefulness of the genetic approach is vitiated inasmuch as we cannot distinguish these several genotypes.[12]

In the present state of knowledge we are limited to a human taxonomy based upon a dozen or so human characteristics. This number is insignificant when compared with estimates of the total number of genic loci for the human species given by several authors. Boyd[3] and Spuhler[17] estimated this number at 20,000 to 40,000, while Stern[19] estimated a range from 5,000 to 120,000. Boyd[3] has stated that with the present limits of knowledge the genetic differences between individuals of the same race can often be demonstrated to be as large as 10 to 20 genes.

A classification based upon the characteristics which are genetically determined should indicate, among other things, some of the evolutionary processes of the human race.[13, 20] Any anatomic variations which may be chosen as suitable for the purpose of contributing to an analysis of human genetics must satisfy several criteria. They should be objective; they should be qualitative rather than quantitative; their heritability should be demonstrable; they should be "adaptively neutral," as set forth by Dobzhansky,[6] in the sense of being slightly, or not at all, modifiable, and have no discernible adaptive value as well as being relatively stable with regard to mutation rates.

The mandibular second premolar has been chosen as the subject of this study because it satisfies the criteria enumerated. Variations in the pattern of the occlusal surface may be identified and classified. These variations are qualitative. The data which are presented demonstrate the heritability of certain of the variations. The pattern differences of the mandibular second premolar which have been observed are of such nature that the burden of proof must fall upon those who would assert that selective advantage is conferred by the possession of one or any combination of such traits. The ontogeny of the occlusal crown surface is such as to exclude the influence of exogenous factors upon the hereditary anlage.

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The objectives of this study are twofold: first, to isolate and define the common morphologic traits of the occlusal surface of the mandibular second premolar; second, to demonstrate the inheritance of these traits. Analysis of the dental casts of a number of identical twins was considered significant in this regard.

REVIEW OF THE LITERATURE

The details of the gross and finer structural modifications of the occlusal crown surface of the tooth under study have been matters of interest to a number of investigators. Most observers recognize the constancy of certain features in the morphologic structures as well as their qualitative variability. They do not, however, describe these variations in sufficient detail for purposes of classification. Except for a few instances, their descriptions were not formulated with the objective in mind of analyzing the relative frequency of the traits.

Wortman[21] referred to the buccal and lingual cusps, the sagittal sulcus, the median ridge of the buccal cusp with its attendant variations, the mesial and distal fossae, and the subdivision of the lingual cusp, but did not give a precise statement of the explicit relationships which obtain between these various components. Black[2] observed the transverse ridge, the mesial and distal fossae, 2 and 3 cusps, and the sagittal sulcus both straight and interrupted. These formations and their relationships are not sufficiently described for the purposes of the present study. Hrdlicka,[8] translating Zuckerkandl,[22] mentioned 2 cusps, the transverse ridge, and the mesial and distal fossae. This is an inadequate description and does not express the wide range of variation in the tooth. Hrdlicka,[8] translating Mühlreiter,[14] credited him with noting the median ridge and the presence of a secondary occlusal ridge. Hrdlicka[8] described the median ridge, accessory mesial and distal ridges, and variations of the median ridge. These features by no means exhaust the range of variation in the mandibular second premolar.

The median occlusal ridge of the buccal cusp is one of the structures with which this study is concerned. Because of its constant occurrence it has been chosen as a point of reference in defining other traits. Hrdlicka[8] stated, "The median of the intermarginal ridges of a crown or a cusp is without exception the best developed and most frequent. It is the axial ridge of the 'protocone' or 'main lobe,' and the main strengthening ridge of the crown or a cusp as a whole."

Pedersen[15] described the lower second premolar of East Greenland Eskimos as consisting of 2 types—one with 2 main cusps, the other with 3. Pedersen also mentioned other investigators (Becker,[1] Hjelmann,[7] de Terra,[4] and Kajava[9]) who have observed the incidence of 2 or 3 cusps among different populations. De Jonge-Cohen[5] recognized 2 basic forms of the lower second premolar, one sexitubercular, the other quintitubercular.

Repeated references to certain characteristics of the occlusal crown surface were noted in the literature. The descriptions of the various structural

modifications of the crown surface pattern by the authors cited are not intended for, nor are they in most cases, amenable to statistical interpretation. In the interests of statistical expediency it is necessary to describe these characteristics in terms which are not ambiguous.

DATA

All of the casts and teeth upon which the observations of this study are based are from the dental collection of the Laboratory of Physical Anthropology of the University of Arizona. The casts in this collection were obtained by Dr. Bertram S. Kraus. Many of the casts are those of the dentitions of children in the Tucson, Arizona, Public Schools; others represent family pedigrees. Some of the casts represent native populations, for example, those taken at the San Xavier Papago Indian Reservation and at Pascua, a Yaqui village near Tucson.

The technic employed in making impressions and casts followed standard practice among dental technicians, but in many cases was modified to meet the exigencies of circumstance. The impressions were made with an alginate material and the casts poured in dental stone.

The composition of the sample by ethnic group is as follows:

Ethnic Group	*Number of Teeth*
Unrelated Caucasoid	69
Dizygotic twins (Caucasoid)	47
Monozygotic twins (Caucasoid)	57
Papago	44
Chinese	24
Negroid	36
Mexican	42
Mexican-Yaqui	85
Prehistoric Indian	48
Total	452

The prehistoric Indian material is from the catalogued collection of the Arizona State Museum, courtesy of the Director, Dr. Emil W. Haury, and represents material from various prehistoric sites throughout Arizona.

Although more than 800 casts are available for study in the Laboratory of Physical Anthropology, only 452 were used. This was due to several factors: A number of the casts were those of deciduous teeth; other casts in certain instances lacked the lower second premolar; the patterns may have been obscured by dental work or caries, or wear of the occlusal surface may have obscured the traits.

The mandibular second premolar erupts between the eleventh and twelfth year of life.[16] Except in cases of severe attrition due to malocclusion or coarse diet, the first signs of wear should not appear until the beginning of adult life, possibly around 20 years of age. The major portion of the sample falls between the ages of 10 and 20 years. Many specimens included in the sample are those of individuals considerably older. In normal occlusion the tooth most frequently presents the peripheral surfaces of its occlusal area

for attrition. In each sample the only consideration was whether the tooth clearly presented the traits in question. The sample was selective in that those teeth which did not were rejected.

METHODOLOGY

The morphology of the mandibular second premolar is variable. The range of variation was exploited to discern the possible relationships which might obtain between components of the occlusal crown surface of the tooth. An intensive study made possible the isolation of certain variations which, though common, might indicate varying frequencies of occurrence. The results of this investigation and the literature revealed certain relationships and discrete features which appeared promising for further study.

An outstanding morphologic feature of each mandibular second premolar was the median occlusal ridge of the buccal cusp. The median ridge is always prominent and dominates the occlusal area of the tooth. The constancy of this feature justifies its selection as a reference point for a number of variations in the occlusal crown pattern. The character of the median occlusal ridge itself was observed to be subject to a number of variations. Two of the most common variants were chosen as traits.

Certain additional significant variations of the occlusal surface appeared with high frequency in most of the samples studied. Accessory occulsal ridges, if present, bore certain definite relationships to the median occlusal ridge. Each mandibular second premolar bears 2 more or less distinct fossae, one mesial to the median ridge of the buccal cusp and the other distal thereto and in almost every specimen at a distinctly lower level. The distal fossa, as has been pointed out by Wortman[21] and later authors, forms a valuable guide in determining the mesial and distal surfaces of the extracted tooth and the side of the jaw to which it belongs. The feature is so constant in its appearance and exhibits so little variation that it was excluded from consideration. The sulcus which runs in a mesiodistal direction is subject to a number of variations, 2 of which were chosen as distinctive patterns with a high frequency of occurrence.

The lingual lobe presents a number of differing relationships to the long axis of the median occlusal ridge of the buccal cusp. A fairly common characteristic of the lingual cusp was its division into double or, less commonly, triple cusps. Where the lingual cusps are divided a special set of relationships becomes evident between their disposition and the long axis of the median ridge of the buccal cusp.

These several variations all represent fairly common and discrete patterns which exist on the occlusal surface of the tooth alone. Other characteristics observed were the grooving of the lingual and buccal vertical surfaces of the tooth but these are not included in the present study because of the difficulty involved in observing them on dental casts.

Following the preliminary survey a list was made of those traits which were sharply defined and which were subject to fluctuation in frequency. A list was compiled which included each tooth suitable for observation. The

tooth was identified as to the ethnic group to which it belonged and was inspected for those traits defined in this study. It then became possible to plot the occurrence of the specific traits by ethnic group. The ethnic breakdown is based upon an earlier study concerned with traits of the mandibular first premolar by Kraus and Furr.[11] The ethnic groups were established as follows: The Caucasoid included unrelated samples as well as dizygotic and monozygotic twins. The monozygotic twins were used to increase the size of the sample, but to avoid biasing the sample only one individual of a pair was used. The Mongoloid included Papago, Prehistoric Indian, and Chinese populations. The Negroid was not further broken down. The Caucaso-Mongoloid included Mexican and Mexican-Yaqui populations.

DEFINITION OF TRAITS

Trait 1. The Occlusal Ridges of the Buccal Cusp.—

Ordinarily the buccal cusp shows a prominent and steep median ridge on its occlusal surface. However, many of the teeth examined have an accessory ridge or ridges which follow a course roughly parallel to the median ridge, but considerably shorter in length. If there is only one additional ridge, it may lie either mesial or distal to the median ridge. In those instances where two additional ridges are present, one is mesial to, and the other distal to the median ridge. The trait has four distinct aspects:

1A. Median occlusal buccal ridge only (Fig. 1*A*).
1B. Median occlusal buccal ridge, plus one distal ridge (Fig. 1*B*).
1C. Median occlusal buccal ridge, plus one mesial ridge (Fig. 1*C*).
1D. Median occlusal buccal ridge, plus mesial and distal ridges (Fig. 1*D*).

Trait 2. The Median Occlusal Ridge of the Buccal Cusp.—

All mandibular second premolars examined had, on their occlusal surface, a more or less prominent median ridge running from the apex to the base of the buccal cusp. In sagittal cross-section this ridge shows a considerable variation of form. The commonest condition was that of the ridge having a uniform appearance throughout its course. Less commonly the form of the ridge changed in the area near its base. In that area the ridge diverged and formed two or more secondary ridges. It is therefore possible to delineate two aspects of the trait:

2A. The median ridge is single (Fig. 2*A*).
2B. The median ridge is divergent (Fig. 2*B*).

Trait 3. The Relative Position of the Lingual Cusp.—

In those instances in which there is a single lingual cusp, the greater volume of its mass, as viewed from its occlusal aspect, may be related in one of three ways to the long axis of the median occlusal ridge of the buccal cusp. Therefore, three aspects of the trait are evident:

3A. If the larger portion of the lingual cusp is medial to the long axis of the median ridge of the buccal cusp, the position is described as medial (Fig. 3*A*).

3B. If the larger portion of the lingual cusp is mesial to the long axis of the median ridge, the position is mesial (Fig. 3*B*).

3C. If the larger portion of the lingual cusp is distal to the long axis of the median ridge, the position is described as distal (Fig. 3*C*). In order to observe the various aspects of this trait accurately it is necessary to orient the lingual aspect of the tooth in such a manner that it is in direct opposition to, or at right angles to, the mesiodistal plane of the tooth.

Trait 4. The Number of Lingual Cusps.—

In accordance with a practice established by Kraus,[10] a cusp is defined as having an independent apex, however slight. The apex represents an elevation which is readily distinguished and may be palpated. One lingual cusp was the commonest condition. Less frequently there were two. Three lingual cusps were relatively rare. Four lingual cusps were not observed. Thus, in this sample the trait presents three aspects:

4A. One lingual cusp (Fig. 4*A*).

4B. Two lingual cusps (Fig. 4*B*).

4C. Three lingual cusps (Fig. 4*C*).

Trait 5. The Independence of the Lingual Cusp.—

The morphologic relationships existing between the buccal cusp and the lingual cusp are varied. In most cases the lingual cusp possesses an apex which is independent of the buccal cusp. However, when the lingual cusp is joined or fused with the central occlusal ridge of the buccal cusp certain relationships become evident. The tooth, in such instances, presents a cuspoid appearance. The sagittal sulcus is interrupted and the apex of the lingual cusp cannot be palpated. The area of the fused lingual cusp which would mark the occlusal ridge of that cusp is continuous with the median ridge of the buccal cusp. Thus, the median occlusal ridge of the buccal cusp runs in an unbroken line to the junction of the occlusal and lingual areas of the tooth. Hence, two categories are therefore designated for this trait:

5A. The lingual cusp is independent (Fig. 5*A*).

5B. The lingual cusp is fused with the median occlusal ridge of the buccal cusp (Fig. 5*B*).

Trait 6. The Position of Multiple Lingual Cusps.—

In cases where double or triple lingual cusps occur, several relationships may exist between the buccal cusp and the lingual cusps. These relationships may be described in terms of the position of the lingual cusps with respect to the long axis of the median occlusal ridge of the buccal cusp. The lingual cusps converge toward a point either directly opposed to the base of the median occlusal ridge or to a point which lies mesial or distal to it. Thus, three aspects of the trait occur:

6A. The lingual cusps are medial to the median occlusal ridge of the buccal cusp and a horizontal extension of the long axis of the median ridge would bisect the total mass of the lingual cusps (Fig. 6*A*).

6B. The greater part of the total mass of the lingual cusps is mesial to the median occlusal ridge of the buccal cusp (Fig. 6*B*).

6C. The greater part of the total mass of the lingual cusps is distal to the median occlusal ridge of the buccal cusp (Fig. 6*C*).

Trait 7. The Sagittal Sulcus.—

The sagittal sulcus follows a mesiodistal course between the buccal cusp and the lingual cusp. Although a number of individual variations relating to the patterns formed by the sulcus might be described as distinguishing one tooth from another, for the purposes of this analysis, two aspects of the general pattern of the sulcus were considered.

Figs. 1 through 7.

7A. The mesiodistal course of the sulcus is not interrupted by any enamel extensions from the occlusal medial ridges of buccal or lingual cusps (Fig. 7*A*).

7B. The sulcus may be interrupted in its course by elevations or by enamel extensions projecting from the occlusal surfaces of the buccal and/or the lingual

TABLE I

RELATIVE FREQUENCIES OF ASPECTS OF THE TRAITS BY ETHNIC GROUP AND SUBGROUP (TRAIT FREQUENCY IN PER CENT)

ETHNIC GROUP / SUBGROUP	1				2		3			4			5		6			7	
	A	B	C	D	A	B	A	B	C	A	B	C	A	B	A	B	C	A	B
Caucasoid	49.6	36.4	1.4	12.6	86.8	13.2	37.6	54.1	8.3	60.1	39.2	.7	100.0	.0	44.6	5.4	50.0	68.7	31.3
Unrelated	43.5	39.1	2.9	14.5	87.0	13.0	25.0	64.6	10.4	69.6	30.4	.0	100.0	.0	47.6	4.8	47.6	65.2	34.8
Dizygotic	55.3	34.0	.0	10.7	91.5	8.5	50.0	42.3	7.7	55.3	44.7	.0	100.0	.0	57.1	4.9	38.0	61.7	38.3
Monozygotic	55.6	33.3	.0	11.1	78.5	21.5	62.5	37.5	.0	42.9	51.8	5.3	100.0	.0	21.5	7.1	71.4	89.3	10.7
Mongoloid	7.0	39.5	8.8	44.7	82.8	17.2	54.6	43.4	2.0	91.0	9.0	.0	96.6	3.4	20.0	.0	80.0	16.4	83.6
Papago	7.1	28.6	7.1	57.2	95.5	4.5	45.0	52.5	2.5	97.6	2.4	.0	93.2	6.8	.0	.0	100.0	6.8	93.2
Prehistoric Indian	8.3	33.3	10.4	48.0	81.3	18.7	59.5	38.1	2.4	95.7	4.3	.0	97.9	2.1	50.0	.0	50.0	6.2	93.8
Chinese	4.2	70.8	8.3	16.7	62.5	37.5	64.7	35.3	.0	70.8	29.2	.0	100.0	.0	14.3	.0	85.7	54.2	45.8
Negroid	33.3	36.1	5.6	25.0	77.8	22.2	21.0	79.0	.0	52.8	41.7	5.5	100.0	.0	.0	26.7	73.3	72.2	27.8
Caucaso-Mongoloid	41.7	38.6	.0	19.7	81.1	18.9	37.0	61.6	1.4	65.2	34.8	.0	94.5	5.5	28.2	20.5	51.3	45.7	54.3
Mexican	42.9	50.0	.0	7.1	85.7	14.3	20.7	75.9	3.4	76.3	23.7	.0	100.0	.0	44.4	11.2	44.4	45.2	54.8
Mexican-Yanqui	41.2	32.9	.0	25.9	78.8	21.2	47.7	52.3	.0	59.5	40.5	.0	91.8	8.2	23.3	23.3	53.4	45.9	54.1

cusps. In other cases there may be a banking up of the medial ridges of either or both the buccal and lingual cusps. Therefore, the mesial and distal fossae are not joined in one continuous sulcus but are two distinct depressions (Fig. 7*B*).

Observations of the frequencies of aspects of the traits are shown in Table I.

TWIN STUDY

In a sample of 145 Caucasoid teeth the frequencies of the various aspects of the traits are as shown in Table II. Column III in Table II is calculated on the principle that if the probability of an event is p, then the probability of the event occurring twice in 2 trials is p^2. The probability of any 2 of the teeth being alike in any of the various aspects of a given trait is the sum of the squares of the frequencies of each. In this study such a similarity is designated a concordance and the expected frequencies of such concordances for each trait are shown in Column III of Table II. Two comparisons, one on the left and one on the right, were possible with each pair. Each of these comparisons was either a concordance for a given trait or it was not.

TABLE II

ASPECT FREQUENCIES AND EXPECTED CONCORDANCES

I	II OBSERVED FREQUENCY				III
TRAIT	A	B	C	D	EXPECTED PER CENT OF CONCORDANCES
1	49.6	36.4	1.4	12.6	39.5
2	86.8	13.2	–	–	77.1
3	37.6	54.1	8.3	–	44.1
4	60.1	39.2	.7	–	51.5
5	100.0	.0	–	–	100.0
6	44.6	5.4	50.0	–	45.2
7	68.7	31.3	–	–	57.0

Tables III and IV show the results of comparisons for concordance for the monozygotic and dizygotic twin series in terms of their difference in structure from the total representative Caucasoid population.

TABLE III

COMPARISON OF OBSERVED WITH EXPECTED CONCORDANCE IN MONOZYGOTIC TWINS

TRAIT	X^2 (1 d.f.)*	P VALUE	
		LESS THAN	MORE THAN
1	32.4	.001	—
2	7.7	.01	.001
3	9.2	.01	.001
4	21.8	.001	—
5	—	—	—
6	12.2	.001	—
7	13.9	.001	—

*Computed by formula:

$$X^2 = \Sigma \left[\frac{(|X - \phi| - k)^2}{\phi} \right].$$

Where ϕ = expected number of concordances or discordances.
X = observed number of concordances or discordances.
k = Yates' correction for continuity (0.5).

Table III for the monozygotic series indicates all of these traits, with the exception of one whose constant incidence in the Caucasoid population precluded its statistical use, were demonstrated to be present to a degree which significantly exceeded what chance alone would permit. The findings are, therefore, consistent with the interpretation that these traits are regulated by inheritance.

Table IV for the dizygotic series indicates none of the traits in this sample shows a significant difference in concordance from that which the operation of chance would permit.

TABLE IV

COMPARISON OF OBSERVED WITH EXPECTED CONCORDANCE IN DIZYGOTIC TWINS

TRAIT	X^2 (1 d.f.)	P VALUE		
		LESS THAN		MORE THAN
1	3.6	.10		.05
2	.14		.70	
3	.00	—		.99
4	1.2	.30		.20
5	—		—	
6	.64	.50		.30
7	2.04	.20		.10

The question may arise concerning the lack of complete and perfect concordance among the monozygotic twin pairs. Spuhler[18] accounted for this difference as the result of the action of nongenetic environmental factors with the genotype remaining constant.

If the hypothesis relative to the inheritance of the traits discussed in this study is valid, we can expect the monozygotic pairs to differ significantly from the total representative Caucasoid population. This has been demonstrated in this analysis. Likewise, if the hypothesis is valid, we should not expect the dizygotic pairs to differ significantly from the representative population. This situation is apparent in all instances and is in accord with the concept that the genes of each of a dizygotic pair are inherited independently of the other.

CONCLUSIONS

Seven morphologic traits embracing 19 aspects of the mandibular second premolar have been characterized. These traits occurred in 4 ethnic groups: Caucasoid, Mongoloid, Negroid, and Caucaso-Mongoloid. Although the traits and most of their variants appeared in each ethnic group, their relative frequency varied from group to group. The differing frequencies suggest the possibility of determining ethnic affinity by an analysis of the dental traits.

The basis in inheritance has been indicated by an analysis of 17 monozygotic twin pairs. Results of the analysis showed a significantly higher concordance, or similarity, in these monozygotic twin pairs than found in the total representative Caucasoid population. The same traits when compared in 12 dizygotic pairs of twins did not differ significantly in their concordance from that which would occur in the total representative sample.

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DENTAL AUTORADIOGRAPHY
I. CHOICE OF RADIOISOTOPE

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AN earlier publication dealt with posteruptive changes of teeth in relation to the resistance of the tooth to decay.[1] Radioisotope uptake was used as a tool for investigation in many of the studies discussed. During the past two years, in continuation of earlier studies of posteruptive changes, autoradiography has been used to supplement other histopathologic technics for the study of the structure of extracted teeth. A simple and satisfactory method for the purpose has been developed, and the findings from its use have proved valuable. They offer information regarding the permeability of the tooth to aqueous solutions in greater measure than that which is available from the usual staining and photographic methods. The technics employed already have been described in general terms by others,[2] or are adaptations of common procedures. Yet believing that a description of our methods and findings will be of value to other workers in related fields of investigation, it is planned to describe them in a series of brief presentations, each dealing with specific aspects of the autoradiographic recording of dental findings. The current report will deal with general methodology and with the basis for the choice of specific radioisotopes as tools for study.

Searching for an improved method for the measurement of dentinal sclerosis, it was planned to use the capacity for radioisotope uptake as a measure of the internal space of the tooth. To avoid uptake secondary to ion exchange, radioiodine first was used. For purpose of comparison, some autoradiograms also were made using P^{32}, recognizing that this element would become fixed within the dental structure through ion exchange as well as through adsorption.

Neither the radioiodine nor the radiophosphorus gave autoradiograms with sharp histologic detail. It was reasoned that the blurring that might be expected from the emission of long range beta particles or gamma rays from the lower layers of the dental structure would serve to obscure the registry on the film of the radiant energy being emitted from the surface of the specimen. As a consequence, radiosulfur was tried. This isotope gives off its radiant energy only in the form of beta particles, and the range of these particles in the case of radiosulfur is slight; they can penetrate a maximum thickness of only 0.2 mm. of water, and about half that thickness of dentin. The autoradiograms obtained through the use of S^{35} are markedly sharper in

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definition than those obtained when either of the other two isotopes was used. Thus S^{35} is superior for the portrayal of the surface characteristics of the sample. However, it should be emphasized that each of the 3 isotopes used served to portray different characteristics of tooth structure, and thus each provides a different approach to the study of dental substance.

TECHNIC

Preliminary to study, tooth specimens were split longitudinally in the buccolingual plane using a diamond-charged rotary saw. Half of each tooth customarily was preserved for future reference. The half to be examined was polished to a plane surface free from scratches through the use of a fabric-covered lapidary wheel.* For rough polishing the wheel was charged with an aqueous slurry of 600 grit aluminum oxide powder. Then levigated alumina was used, and the final polishing was done on an uncharged felt-covered disc. After washing with a soft brush and drying, the specimen was weighed for future reference, then photographed first unstained and then after staining, either with basic fuchsin or with silver nitrate. In some specimens, the staining process was delayed until after the autoradiogram had been made.

The radioisotope gained entry to the specimen through imbibition. A small plastic planchet was used as a container for the specimen and the isotope solution, and the planchet in turn was kept in a covered Petri dish to prevent evaporation. The polished surface of the tooth was placed downward on the planchet, with a small bent piece of fine glass rod interposed between it and the planchet so as to permit contact of the solution with the entire face of the specimen. Then the isotope solution was added from a capillary tube until the cut surface was submerged, from 1 to 2 ml. of solution being sufficient. When the specimen had remained immersed for 18 to 24 hours, it was removed from solution, rinsed in running water, blotted, rubbed gently with facial tissue to loosen any surface deposit, and then dried. The strength of the isotope solution was designed to be such that the specimen when treated as described above would yield satisfactory exposure to the film through 24 hours of contact with the latter. For radioiodine, radiophosphorus and radiosulfur, the amount of isotope needed for each milliliter of solution was 5, 2, and 10 microcuries, respectively.†

In order to maintain the radioactive tooth specimen in exact registry with the photographic film for the necessary period of time, a device was used similar to that illustrated in Fig. 1. This provides (a) a flanged base on which the strip of 35 mm. film rests, and (b) a felt-faced mounting block to which the tooth specimen is attached and which serves to orient the specimen accurately in contact with the film surface when the device is assembled. The device permits the simultaneous exposure of several specimens if desired. To assemble, the unpolished surface of the tooth is fastened to the felt surface of the mounting block in such a manner that the cut surface can assume a position plano-parallel with the face of the film. Duco cement has proved useful

*Eberbach adjustable portable polisher, E. Machlett and Son, New York City.
†The isotopes were obtained from Abbott Laboratories: no carrier was added.

for this purpose. When the cement has dried thoroughly, the specimen is ready for mounting on the film. In complete darkness, a piece of 35 mm. film is cut slightly longer than the face of the base block and is placed, emulsion surface uppermost, on the base. The mounting block then is lowered over the film in such a manner that good registry between the tooth and the film will be assured. The mounting block then is bound to the base block through the use of rubber bands at each end of the assembly (*C*). As an added precaution against exposure to light, the assembly is enclosed in a telescoping cardboard box, and the whole then stored in a light-tight bin in the darkroom. There it remains until the desired exposure time has elapsed. Then, still in total darkness, the film is transferred to a daylight type developing tank and is

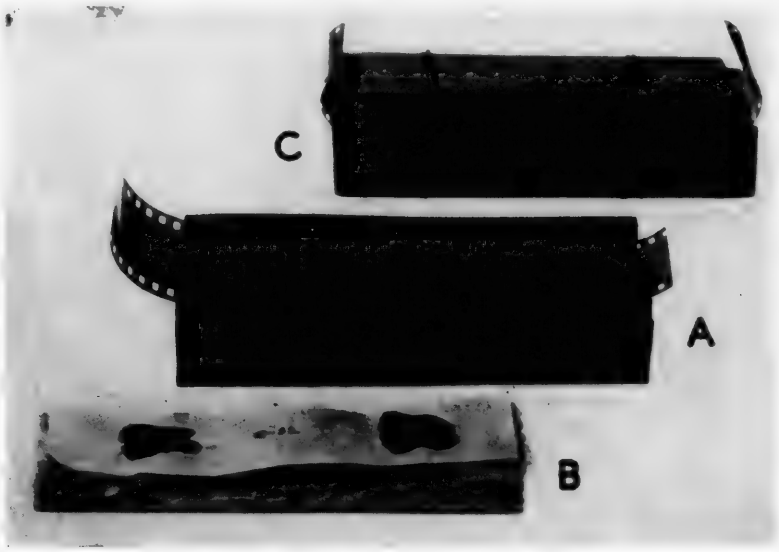

Fig. 1.—Cassette for the autoradiography of teeth.

processed in the manner recommended for photographic purposes for the emulsion which has been used. For the autoradiograms illustrated in this report, Plus-X film was used. It has seemed important to keep the developing tank continuously agitated during the process of developing and fixing the film. For that purpose, a platform type of laboratory shaker has been employed.* For photographic enlargement of the autoradiogram, an intermediate positive must be made in order to show the intensity of uptake as a function of the darkening of the film.

RESULTS

Fig. 2 shows a photographic image of the split tooth. This specimen was mounted in Bakelite, a procedure which we have discontinued. One portion

*Eberbach Laboratory Shaker, variable speed.

has been stained with basic fuchsin. Fig. 3 shows the autoradiogram obtained after the specimen had been immersed in radioiodine. Figs. 4 and 5 similarly are from autoradiograms made after immersion in solutions of radiophosphorus and radiosulfur, respectively. Between each treatment with isotope, the specimen had been washed for long periods of time to elute the isotope previously employed.

Fig. 2.—Photograph of stained and unstained halves of the tooth used for autoradiography.

Similarities will be noted between the various autoradiograms and the photograph, yet each likewise presents distinctive characteristics. Without going into analysis of the regional findings, it will be noted that cementum, dentin, and enamel differ, respectively, in their avidity for each of the isotopes: cementum takes up the most, enamel the least. There was less differentiation between uptake by the 3 tissues from phosphorus than from either of the other 2 isotopes.

The sulfur autoradiogram shows the greatest detail of histologic structure. Its pattern resembles most closely that shown in the photographic image of the stained section, yet is sharper and has greater contrast than does the latter. One can visualize the individual dentinal tubular tracts, and can observe accentuation of isotope uptake at the dentinoenamel junction and within regions of the enamel.

Attention is directed toward the different pattern of the autoradiograms at the root of the tooth. It will be noted from the photograph that a root tip has been cut diagonally, exposing the root canal. The root tip shows cementum at its periphery. One may properly assume that an envelope of

cementum likewise underlies the root tip, and that such cementum has taken up isotope in each instance in measure greater than has the dentin. The sulfur autoradiogram shows the limits of the root canal and of the cementum exposed at the surface of the specimen as a sharply delimited region of high isotope uptake, appearing black in the illustration. The autoradiograms made with iodine and with phosphorus, on the other hand, reveal in addition the underlying layer of cementum. This distinction between the different autoradiograms is dependent on the range of the radiant emissions from S^{35} on the one hand, and from I^{131} and from P^{32} on the other. The weak beta emissions from the radiosulfur can penetrate only a tenth of a millimeter of tooth substance,

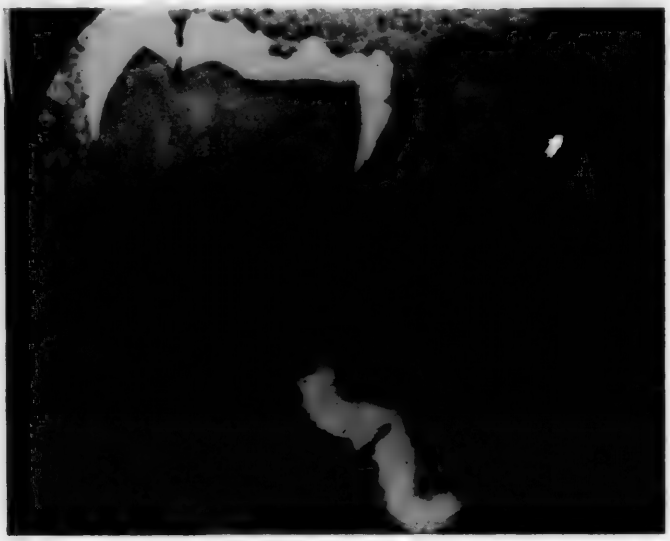

Fig. 3.—Autoradiograph of tooth shown in Fig. 2, after immersion in solution of radioiodine (I^{131}).

and consequently portray surface radioactivity only, even though the deeper tissues may be emitting much radiation. The radioiodine and radiophosphorus, in contrast, have gamma and/or beta emanations with such great penetrability that they reveal not only surface radiation but also that coming from the depths of the specimen.

From the example just cited, one may conclude that sulfur autoradiography permits portrayal of the surface of a specimen of hard tissue, even though the block being studied may be of infinite thickness. This should prove a convenience, if it makes the cutting of thin sections unnecessary. Conversely, the material just described makes it equally evident that the nature of deeper lying tissues may be studied using autoradiographic technics through the use of isotopes which emit radiation of a more penetrating quality. The manner in which these varied autoradiographic technics can best be employed in different studies of hard tissues may be determined by the objectives of the particular investigation.

Fig. 4.

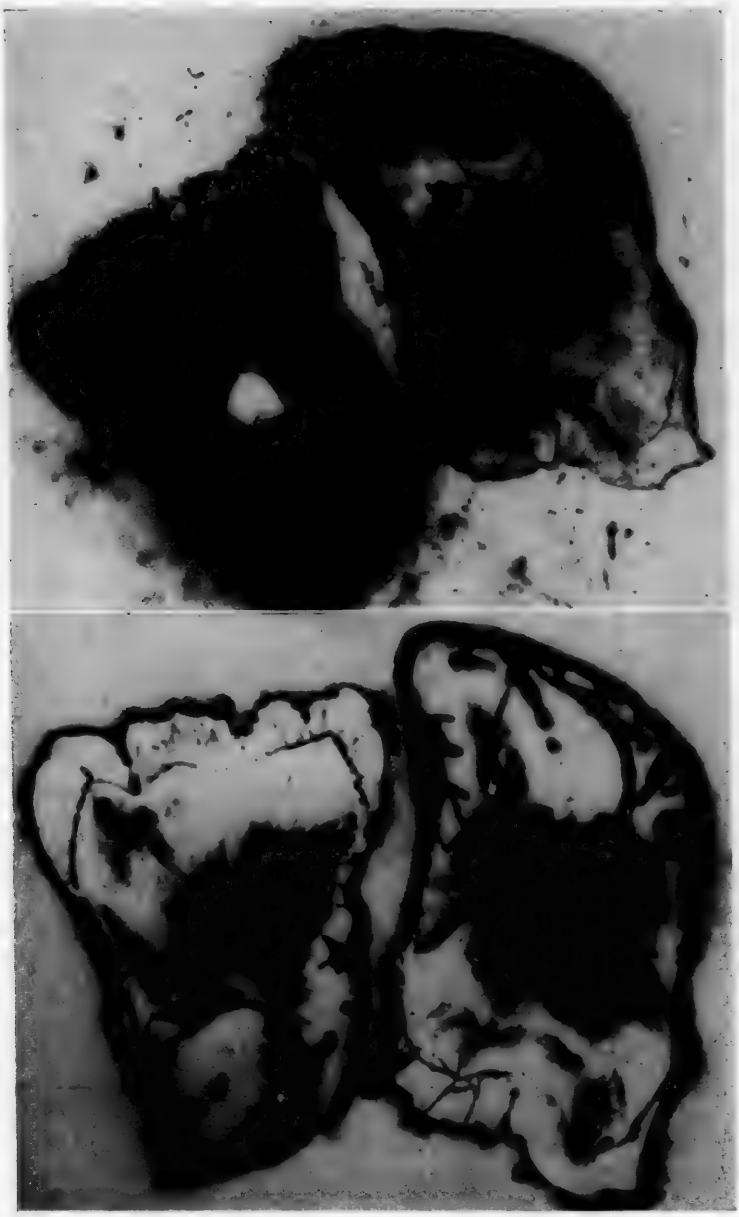

Fig. 5.

Fig. 4.—Autoradiograph of tooth shown in Fig. 2, after immersion in radiophosphorus (P^{32}).

Fig. 5.—Autoradiograph of tooth shown in Fig. 2, after immersion in radiosulfur (S^{35}).

The author wishes to acknowledge the valuable technical assistance of Mr. Lee L. Starrett in the performance of this study, and the photographic counsel and service provided by Mr. Allen E. Weinberg of the Medical Illustration Service in the preparation of the illustrations.

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DENTAL AUTORADIOGRAPHY

II. EFFECT OF PRINTING TECHNICS ON INTERPRETATION

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THE first paper of this series described the technics being used in this laboratory for the autoradiographic study of teeth.[1] The studies in progress are designed to define the posteruptive changes which occur in teeth, and the manner in which these changes may affect the resistance or susceptibility of the tooth to caries. Radioisotope uptake* in vitro by sectioned extracted teeth is being correlated with the pattern and degree of dentinal sclerosis. This is done with the premise that the greater the degree of sclerosis, the less the capacity for uptake of isotope. Autoradiographic findings are being compared and contrasted with findings obtained through the use of histologic and other technics. The advantages of a low-energy beta emitter (e.g., sulfur-35) for visualization of the surface characteristics of the sectioned tooth have been shown.[1]

This report will deal with some technics of film exposure and of photographic printing from the autoradiographic image, and the manner in which variations in these technics may affect the interpretation of the autoradiographic findings.

METHOD

The tooth chosen for this demonstration was an upper second premolar from the mouth of a 65-year-old male patient. On each lateral contact point the tooth showed a chalky area, interpreted as evidence of minor caries of the enamel. The tooth was sawed approximately in half in the buccolingual plane, and the exposed surfaces were polished in the manner previously described.[1] After photographing the unstained cut surfaces, one of the halves was immersed for 24 hours in one milliliter of an aqueous carrier-free solution containing 3.22 microcuries of radiosulfur (S-35) in the form of sulfate ion. Then after slight rinsing, the specimen was dried and all of its surfaces sprayed with a layer of aluminum paint, so as to check irradiation from surfaces other than the polished face. The latter then was freed from aluminum coating through wiping with a cotton swab moistened with acetone, taking care not to disturb the protective film covering the surfaces of the pulp chamber. Then in complete darkness the specimen was placed in contact with

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*The word "uptake" as employed in this presentation refers to the acceptance and retention of isotope by the dental tissue, without connotation as to the manner in which the material is collected or held.

photographic film (Tri-X, Eastman, 35 mm.), successively, for intervals of 1, 4, 8, 24, 48, and 72 hours. Each exposed film was developed using the usual photographic technic. From the various autoradiograms, samples were chosen which showed a suitable range of contrast throughout the various components of the tooth, and these films were used for making photographic prints.

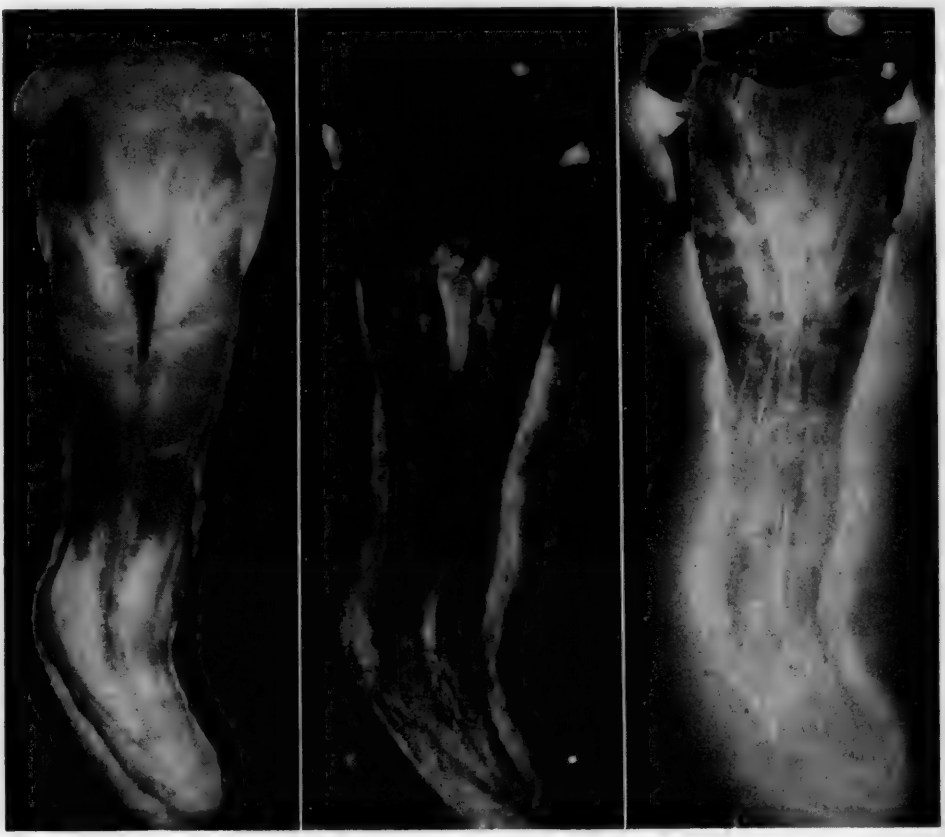

Fig. 1. Fig. 2. Fig. 3.

Fig. 1.—Photograph of polished unstained surface of an upper second premolar tooth. A small portion of the occlusal surface also is shown in the photograph, but it does not show in the illustrations of autoradiograms because it does not come into contact with the film used for autoradiographic registry.

Fig. 2.—Print from radiosulfur autoradiogram of tooth surface shown photographically in Fig. 1. This exposure was made to bring out details in the cementum and dentin. The "halo" extending beyond the limits of the cementum is caused by stray radiation coming from the rough outer surface of the root, where isotope has collected in amounts much greater than on the polished surface. The brilliant spot which appears to be distal to the occlusal surface is explained similarly.

Fig. 3.—Radiosulfur autoradiogram of the same tooth surface, exposed and printed so as to bring out detail in the enamel.

Because of the great difference between the porosity of the enamel, dentin, and cementum, it proved impossible to obtain good rendition of detail for each of the 3 dental components in any single print. Therefore a series of

prints were made, using Varilour paper and Varigam filters (du Pont) to provide a range of contrast, and with exposure time of film image to paper ranging throughout proper limits for the filter being used.

RESULTS

From the collection of prints it was possible to assemble a series showing detail, respectively, in cementum, dentin, and enamel. Even for the dentin in the given tooth, certain characteristics were portrayed better in prints from one length of exposure than from another.

The accompanying illustrations show the photograph of the tooth half (Fig. 1), and prints made from two different autoradiograms, representing, respectively, exposures of the radioactive specimen to the film lasting 4 hours

Fig. 4.—Greater enlargement of crown of the tooth in Figs. 1, 2, and 3 showing pattern of variable uptake beneath the occlusal surface, suggestive of incremental bands.

(Fig. 2) and 3 days (Fig. 3). The former was developed so as to bring out maximum detail in the cementum, whereas the latter was developed with maximum detail of enamel as the objective. Fig. 4 represents a greater enlargement of the crown of the tooth, made from the same autographic film used for Fig. 3.

DISCUSSION

The prints from the autoradiograms show reversal of light and dark from the findings on the autoradiographic film. As a consequence, the regions of maximum radioisotope uptake appear white in the illustrations, those with

no uptake, black. All gradations of pigmentation will be noted. The cementum has taken up the most, the enamel the least isotope, and the dentin shows a wide range of variability of uptake. We may assume from earlier studies that in greatest measure the uptake of the radiosulfur is a function of the porosity of the specific region of the tooth.

It will be noted that a photographic exposure sufficient to give detail in the cementum was inadequate for the enamel and that, conversely, when the exposure was sufficient to provide detail in the enamel, detail in the cementum and in much of the dentin was burned out. Histologic detail will be noted in the dentin which parallels in large measure the optical appearance of the tracts of dentinal tubules seen in the photograph. These distinctions as to dentinal pattern are seen in some degree in each of the autoradiographic prints. The regions of enamel opacity at the contact points show up in each print as regions of marked radioisotope uptake. The fissures in the enamel likewise show accumulation of isotope. In the films made to show enamel detail, lack of uniformity of uptake will be noted, suggesting variability of radioisotope uptake in regions which presumably are neither carious nor fissured. When studied in the enlarged image of the crown, it will be noted that the light areas are arranged in such a manner that they suggest the presence of incremental bands. Likewise, it will be noted that scratches are present in one region. These scratches were not evident in the photograph, and were not discovered until the specimen was observed closely while it was rotated under reflected light.

When it is recalled that the image created through contact of the tooth surface with the film is produced by the discharge of beta particles from the radiosulfur, and that these beta particles can penetrate through only a tenth of a millimeter of tooth substance, it is evident that the autoradiographic image portrays only surface characteristics. It will be understood also that slight irregularities in the polished surface, such as the scratches just mentioned, will retain more isotope than will the polished surface, and consequently will cause artefacts to appear on the finished print.

Mention should be made regarding the appearance of the dentinal tubules underlying the enamel lesion shown at the left-hand side of the photograph. From the photograph one might assume that there was destruction of the peripheral portions of these tubules. If such were the case, that region would show greater porosity and consequently greater uptake of isotope than the adjacent tubules. The same region in the autoradiograms shows that the reverse is true; the tubules in the region described actually show less uptake of isotope than do those adjacent to it. This is interpreted as evidence that the tooth has reacted to the leaky enamel through greater sclerosis of the dentinal tubules tributary from the lesion to the pulp than in the tooth crown as a whole. Similar findings can be noted at the base of the enamel defect on the opposite side. These findings have not been unusual. They are considered as instances of increased resistance to dentinal caries. In contrast, in analogous regions of other teeth where caries still is progressing, the tubular tracts

underlying the lesion have shown increased uptake of isotope, with some degree of isotope rejection (dentinal sclerosis) sometimes being seen in tracts lateral to the lesion. These lateral sclerotic tracts likewise have been considered by us as evidence that the tooth is endeavoring to limit the spread of the caries. These findings will be described more in detail in a future publication.

SUMMARY

Technics employed in the autoradiographic portrayal of tooth structure are described. It is shown that in the study of hard tissues such as the tooth, it is advantageous to make a series of exposures of the radioactive specimen of progressively longer duration to the photographic film, and likewise to make photographic prints from the various autoradiograms of similarly variable exposure times and of contrast, so as to bring out, in the different prints, the detail afforded by areas of varied porosity. Attention is drawn to the parallelism between optical characteristics as seen in the photograph and those seen in the print from the autoradiogram. It is shown that regions which photographically might suggest dentinal destruction may instead be revealed in the autoradiograms as areas of increased resistance to decay.

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COMPRESSION OF TEETH UNDER THE LOAD OF CHEWING

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DURING mastication the teeth are subjected to intermittent pressure. The amount of the load, both under normal masticating force and under maximum biting effort, varies in different human societies and individually according to chewing habits and type of foods consumed. The maximum biting force between molars was found to be about 75 to 150 lb. in a group of American adults,[1] and 200 to 300 lb. and more in Eskimos on their original native diet.[2]

Much of the load on the teeth is being transmitted from the occlusal surface through a rather thin outer cylinder of enamel which is more resistant to compression than the inner dental tissues. Due to the conical shape of the root which distributes the forces over a wide area, the actual pressure concentrated in the enamel per unit of area is of a much higher order than the pressure exerted on the tissues underlying the teeth. In each point of contact of the root with its socket, only a fraction of the vertical pressure is being transmitted to the periapical tissues, according to the parallelogram of forces.

The question raised for consideration in the present study is whether the application of pulsating loads within physiologic limits has any measurable effects on the teeth. The biological, chemical, and physical impact of compression on the conglomerate microstructure of the tooth is a vast and complex field which has received little if any attention.

EFFECTS OF PRESSURE IN GENERAL

The reactions of tissues under pressure can be divided into three broad classifications: (1) Physical reorientation and molecular relocation, (2) modifications of diffusion processes and of chemical reactions, and (3) adaptive biologic processes.

Many biologic and physical effects are known to occur solely under the influence of pressure, and numerous observations compel the conclusion that pressure exerts specific effects on the nature and velocity of biochemical processes.[3] The reaction of proteins may be modified and they even may be coagulated with the action of pressure alone, at room temperature.[4] Compression may produce reversible or irreversible reactions in organic compounds, influencing both polymerization and saponification, as well as the inversion of cane sugar. Micro-organisms are killed at different pressures, thus different organisms vary greatly in their resistance to the action of pressure.[5]

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Most tissues, in particular skin, bone, and vascular walls, are exceedingly sensitive to pressure with prompt adaptive physiologic or pathologic responses to variations in the load.

In the case of the dental tissues, the problem hinges principally on whether the teeth are compressible under pressures exerted in the ordinary act of chewing, and whether they are affected differently at greater or lesser compression within physiologic limits, or under stresses which may reasonably be encountered.

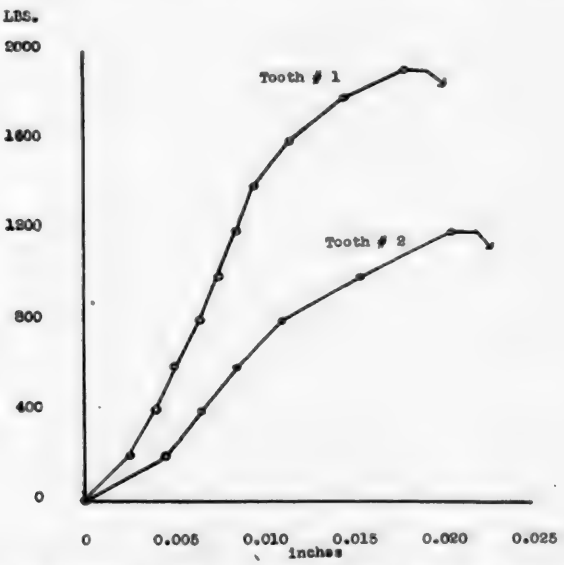

Fig. 1.—Compressibility of teeth under load. The curves show the width in the variability, compressing different human molars to the point of fracture. (The softer underlying tissues in the mouth do not reduce shortening of the tooth under a given load. To illustrate: a scale registers weights from approachment of its platforms. This compressive shortening, expressed as weight, remains the same, whether the scale stands on concrete, on cork, or on a float in water.)

Measurement of shortening under load can be rather accurately done and seemed to be an applicable method for the purpose. If the molar crown is compressible under the relatively low loads physiologically encountered, then changes both in electrical resistance and in the x-ray diffraction pattern can be expected, their demonstration depending mainly on the limitations of sensitivity of the instruments used and in difficulties of experimental arrangements.

References in the literature regarding isotope uptake under varying compression stress are mostly observations in the field of physics. Our own experiments on phosphorus uptake of teeth under varying chewing loads gave some indications of differences in the ionic exchange depending on the pressure to which the teeth are subjected.[7]

METHOD OF MEASURING COMPRESSIVE SHORTENING

The specimens used were intact, caries-free human molars that were extracted on the day of the tests and kept in saline solution. Seventeen teeth,

each from a different individual, were measured. Two flat parallel surfaces were produced by grinding at the occlusal and apical ends. The teeth were then placed between the plates of a Webster compression testing machine and continuously increasing loads were applied to the parallel ends. The compressibility of the underlying structure, whether steel, bone, or plastic does not materially influence the compressive shortening of the teeth under a definite load, provided that a given pressure ultimately is being transmitted through the specimen.

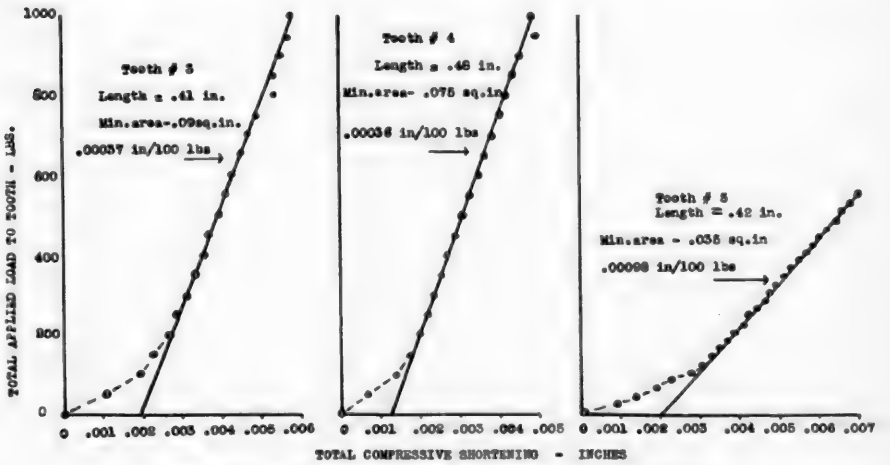

Fig. 2.—Compressibility of human molars under load.

Shortening at the various loads was continuously recorded. Compressibility was relatively much greater at low loads and became remarkably uniform in all cases. The results were very consistent for biologic material. The curves in Fig. 1 portray the general trend of compressibility of teeth under physiologic loads and under larger loads, up to the crushing point. Table I gives the values of compressive shortening for human molars under load.

TABLE I

COMPRESSIVE SHORTENING OF TEETH UNDER LOAD, IN INCHES

TOOTH NO.	100 LB.	200 LB.	300 LB.	400 LB.	500 LB.	600 LB.
1	0.0015	0.0025	0.0035	0.0040	0.0045	0.0050
2	0.0030	0.0045	0.0055	0.0065	0.0080	0.0090
3	0.0020	0.0027	0.0031	0.0035	0.0039	0.0043
4	0.0013	0.0020	0.0023	0.0026	0.0030	0.0034
5	0.0025	0.0037	0.0046	0.0055	0.0064	0.0073
6	0.0015	0.0025	0.0033	0.0040	0.0047	0.0055
7	0.0025	0.0050	0.0070	0.0085	0.0105	0.0125
8	0.0020	0.0035	0.0045	0.0055	0.0065	0.0075
9	0.0025	0.0045	0.0065	0.0080	0.0095	0.0105

As the curves are not comparable without introducing the respective length in sectional areas, Fig. 2 gives these additional data.

The areas given are approximate and the stresses, particularly because of the nonhomogeneous structure, give only an order of magnitude. The modulus of elasticity was determined from the slope of the straight line portion of the curve and represents the stress divided by the strain per unit length.

TABLE II

MODULUS OF ELASTICITY OF HUMAN MOLARS, WITHOUT ROOTS

TOOTH NO.	APPLIED LOAD (LB.)	LENGTH (IN.)	MIN. AREA (SQ. IN.)	STRESS (LB./SQ. IN.)	MODULUS OF ELASTICITY (LB./SQ. IN.)
3	500	.41	.09	5,500	1.2×10^6
4	500	.48	.075	6,650	1.7×10^6
5	500	.42	.035	11,600	1.1×10^6

DISCUSSION

As the tooth is compressible under the ordinary load of chewing, the fundamental enamel structure cannot remain unchanged by the intermittent compression and the crystal surfaces cannot face each other regardless of the pressure exerted on them. There are certain limitations in the motions of individual crystals; restraints are produced by the necessity of each crystal to cohere with all its neighbors and with its organic surroundings. The crystals, being less compressible than the organic matrix, tend to move in different directions. It is impossible to predict the direction and the degree of dislocations and rotation of individual crystals but molecular relocation must take place under the stress of compression. Because of the highly intricate lattice of crystal structure and organic matrix, the problem is too complex for any more than a general solution.

We do not have an explanation for the much higher compressibility at low loads which exceeded by far the slack in the measuring system and may be due to a high initial compressibility of the organic substrate as compared to the apatite crystals.

The reorientation of crystals under stress is a known and undisputed method for altering the strength of materials,[6] including metals and crystalloid structures, both homogeneous and composite. Metallurgists are satisfied that such a phenomenon exists although the theoretical explanations that deal with it are mostly assumptions. In view of the difficulties, which have been present until quite recently, in dealing with relatively simple situations of more or less homogeneous materials, it is evident that we may have to wait for some time for an understanding of all the pressure effects and relations in the much more complicated structure of the teeth.

There have been rapid developments in recent years in our knowledge of the ultramicroscopic enamel structure and of the closely interwoven pattern of organic and inorganic enamel components.[8] Some of the old theories of enamel stability have been abandoned without, however, accepting the probability of other than a physical exchange of ions, depending on the concentration gradient and the incidental presence of ions.

Without considering further at this time the possibility of a biologic enamel response to pressure, physically, the dental tissue that is frequently exposed to a higher intermittent compression is structurally not the same as similar tissue under a fraction of that load. The influence of this factor cannot be ignored in considering the molecular orientation, diffusion processes, and maturation of the teeth.

SUMMARY

The teeth are measureably shortened under loads exerted in the normal act of chewing. This shortening under a given pressure takes place regardless of the compressibility of the underlying structures, and depends on what load is ultimately being transmitted through the tooth. Many physical, biochemical, and biologic effects are known to occur solely under the influence of pressure. As the tooth is compressible under the ordinary load of chewing, the fundamental enamel structure cannot remain unchanged by intermittent compression, i.e., molecular relocation and rotation must take place under the stress. The factor of pressure cannot be overlooked as influencing molecular orientation, diffusion processes, and maturation of the teeth.

The authors wish to acknowledge the technical assistance of Prof. W. J. Krefeld, Dept. of Civil Engineering, Columbia University and the Bell Telephone Company in Murray Hill, N. J.

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EFFECTS OF DIAPHRAGMS ON THE INTENSITY OF RADIATION IN AIR

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MANY articles on roentgenography contain some reference to the fact that the x-ray field should be limited by a diaphragm. It is necessary to limit the field of radiation to as small an area as practical. It is apparent that the diaphragm will limit the field of radiation, but this study was made to determine what other effects, if any, will be produced by a diaphragm.

METHOD

A series of 3 diaphragms was constructed (Fig. 1) so that the apertures of these diaphragms were centered in line with the primary beam of the x-ray machine. An ionization chamber also was placed in the direct line of the x-ray

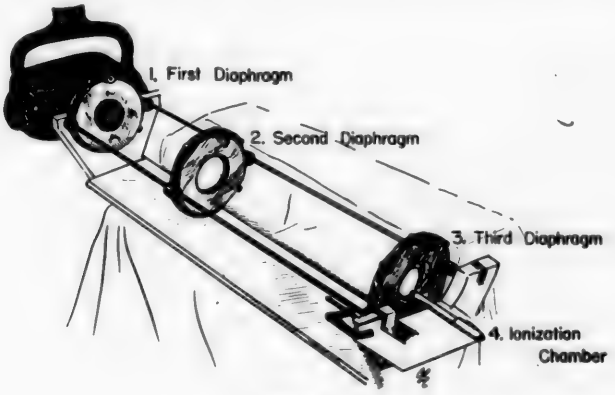

Fig. 1.

beam and centered in line with the diaphragms. Two different settings for the diaphragms were used, one long and one a short distance. In the long distance, the diaphragms were placed 3, 18 and 36 inches from the target of the x-ray tube, and the ionization chamber was placed 38½ inches from the target. In the short distance, the diaphragms were placed 3, 9, and 18 inches from the target of the x-ray machine, and the chamber was placed 19¼ inches from the target. The ionization chamber was set in these experiments so that only the end of the chamber was exposed to the beam of x-ray. The chamber was

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placed in this manner to keep the area of the chamber irradiated at the same level whether a large diaphragm was used or a small diaphragm was used. A standard dental x-ray machine was used with 65 kilovolts and 5 milliamperes. The exposure time used in these readings was one second.

RESULTS

In collecting the data for this experiment, a total of 30 readings was made for each set of diaphragm settings. The average for each set of readings is given in Table I. It will be noted that the figures under long distance were all multiplied by 4, to account for the inverse square law and to make these figures comparable with the same setting under the short distance. Two different diaphragm sizes were used (½ and 3 inches). The size of each diaphragm for each setting will be found under "diaphragm size" in Table I with the diaphragm closest to the x-ray machine as A and the subsequent diaphragms as B and C. The ionization chamber measured only an area of approximately ½ inch in diameter at all times.

TABLE I

	DIAPHRAGM SIZE			LONG DISTANCE	SHORT DISTANCE
	A	B	C		
1.	½	3	½	21.1 × 4 84.4	88.4
2.	½	½	½	21.1 × 4 84.4	90.7
3.	3	3	½	21.2 × 4 84.8	93.0
4.	½	3	3	22.1 × 4 88.4	91.8
5.	½	½	3	22.2 × 4 88.8	93.5
6.	3	3	3	22.7 × 4 90.8	92.3
7.	3	½	3	23.1 × 4 92.4	93.0
	No diaphragm			24.4 × 4 97.6	96.0

There is a general progression from a high reading to a low reading in the change from no diaphragm to very small diaphragms (see Table I). This change in readings can be explained by 2 points. First, as the size of the diaphragm becomes smaller so also does the area of air radiated become smaller; therefore, there is less chance for secondary radiation to be produced. Second, while the major portion of the x-ray beam comes from the focal spot, there is additional radiation coming from all other parts of the x-ray tube. As the size of the diaphragm is made smaller, much of the extra radiation from other parts of the tube is eliminated and therefore cannot add its effect to the reading.

When the short and long readings are compared, it is seen that the short readings are slightly greater than the long readings, which is contrary to the inverse square law. Examining the diaphragm setting for the long and the short readings it is apparent that in the short readings the ionization chamber is closer to the diaphragms than it is in the long readings. Therefore, the secondary radiations from the diaphragms is more intense in the short readings than in the long readings. There is then more radiation reaching the chamber in the short readings, the additional amount coming from the diaphragms. A

similar effect can be seen if reading 1 is compared with 2; if reading 4 is compared with 5; and if reading 6 is compared with 7. In each of these comparisons it can be seen that where diaphragm B is ½ inch the reading is greater than where this same diaphragm is 3 inches. In each of these settings the small diaphragm B is adding secondary radiation which will reach the ionization chamber.

CONCLUSIONS

1. As the field of radiation is made smaller the intensity of the radiation in air will be decreased.

2. The diaphragm can, in itself, add secondary radiation to the field of radiation, and the measurement of this radiation is greater when the diaphragms are close to the chamber.

3. Diaphragms should be used to limit the field of radiation and thereby limit the measurable radiation to the patient. The diaphragms should be placed as close to the source of the x-rays as possible to limit the effect of the secondary radiation from them.

THE USE OF ANTIBIOTICS FOR THE PREVENTION OF BACTEREMIA FOLLOWING ORAL SURGERY

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THE concept that a local infection may act as a focus of dissemination of bacteria has been a point of discussion since the theory of "focal infection" was first offered.[1] Lamb[2] found in 115 cases of subacute bacterial endocarditis that 18 per cent were directly traceable to operative interference with infected foci located in teeth, nasal passages, throats, eyes, and pelvic organs. Since then it has become apparent that the disturbance of a chronic or latent infection may result in a transient or persistent bacteremia. The fact that teeth and adjacent tissues are frequently infected in apparently normal individuals places oral surgery in an important position with respect to possible bacteremia resulting from surgical disturbance of localized infections.

Okell and Elliott[3] found that 60.9 per cent of their patients undergoing oral surgery developed bacteremias. They associated the bacteremia with the severity of gingival disease and the amount of trauma inflicted upon the patient during the operation. In addition, they observed that among 110 patients, having "pyorrheal diseases," more than 10 per cent were found to have a streptococcal bacteremia before any surgical procedures were performed.

Fish and Maclean,[4] showed that the probable source of the bacteria found in bacteremia following oral surgery was located in the gingival sulcus as normal inhabitants of those tissues. Significant confirmation of such views were made by Burket and Burn.[5] In their studies, the gingival margins and crevices were painted with cultures of *Serratia marcescens* before oral surgery was done. Blood cultures collected from these patients showed a significant number of isolations of *Serratia marcescens* following extraction. In 16.9 per cent of the patients, organisms other than the test culture were found. These authors believed that their lower incidence of bacteremia, as compared to the work of Okell and Elliott, was due to the 1:20,000 Adrenalin used as a vasoconstrictor in their procaine anesthetic.

Although the teeth have been incriminated as a focus of infection in bacterial endocarditis, genitourinary infections, and infectious arthritis, it soon

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became evident that not all unexplained infections could be attributed to such foci. Cecil and Angevine[6] studied 200 consecutive cases of rheumatoid arthritis and found that the teeth of these patients were a focus of infection in only 2 cases and a doubtful factor in 11 cases. On the other hand, individual case reports such as that of Bernstein[7] definitely incriminate the oral cavity as a source of bacterial invaders in subacute endocarditis. Pressman and Bender[8] investigated the occurrence of bacteremia following extraction and found that *Streptococcus viridans* was the infecting organism in 87 per cent of their cases. *Streptococcus anhemolyticus* was found in 7 per cent. They concluded that *Streptococcus viridans* gains access to the blood stream through the physical manipulation of a dental focus of infection rather than through the periapical infections of the teeth.

In view of such considerations, the relationship between the bacteria normally present in the oral cavity and its crevices to systemic infection is of primary importance. Infections of the oral cavity, although often mild in origin, have on occasions led to severe or even fatal cases in a significant number of patients. In the past such bacterial disseminations have been difficult to control or prevent. However, with the advent of the sulfonamides and antibiotics, a promise of success for the treatment and prevention of the spread of bacteria from such oral foci is available.

When the present study was undertaken, a wide range of excellent broad spectrum antibiotics were available. Terramycin was somewhat arbitrarily chosen for this research, not because of any known superiority over the other antibiotics but because a supply of "Crystalline Terramycin Hydrochloride"* was made available for our use. In the data on sensitivity testing other antibiotics gave in vitro results equivalent to those found with Terramycin.

Under ordinary circumstances the orally administered Terramycin is rapidly absorbed, yielding therapeutic levels in the patient's blood stream within 3 hours. A single oral dose of 500 mg. maintains a significant blood level for approximately 6 hours. The drug is rapidly excreted by the kidneys and intestines, and the incidence of undesirable reactions is low. A number of articles have appeared concerning the use of this antibiotic in dentistry.[9-12] A review of the literature has failed to disclose sufficient information concerning the sensitivity of various oral invaders to the antibiotics available to dentistry. The present report includes an extensive study of the antibiotic sensitivity of all of the organisms isolated in the blood cultures obtained from these patients along with a statistical analysis of the salient features of this report.

MATERIALS AND METHODS

Since this report compares the incidence of bacteremia in a control group of patients not given Terramycin to a group of patients given a prophylactic dose of Terramycin, the data are considered separately, as well as combined.

*Supplied by Charles Pfizer & Co., Inc., Brooklyn, N. Y.

Selection of Patients.—All patients were obtained from the Department of Oral Surgery of Baylor University College of Dentistry. Only those individuals scheduled for extraction of one or more teeth were used for both the control and prophylactic series. All of these were between 18 and 60 years of age, and no selection was made between sexes.

The 100 patients involved were divided into a control group of 51 and a premedicated series of 49. The two groups were selected at random with respect to the presence of infection and the number of teeth removed. This resulted in a balanced comparison for these factors. The history of each patient included his general health, type of anesthetic given, number and location of teeth removed, reason for removal, radiographic and clinical pathosis, and the results found in the blood cultures. Observations made on the condition of the oral cavity which included the number of caries, presence of periodontal disease, and any pathosis noted around the teeth.

Blood Culture Methods.—Immediately following the surgery (within 2 minutes), the blood sample was taken. As the last tooth was removed, venipuncture was performed and 20 c.c. of blood was aspirated into the sterile syringe. The blood sample was then placed into a sterile Erlenmeyer flask containing 3 c.c. of 4 per cent citrate in saline to prevent clotting. The blood culture was performed as follows: (a) 1 c.c. of blood was placed in a tube containing thioglycollate fluid media for anaerobic study, (b) 3 c.c. of blood was added to 50 c.c. of melted (45° C.) tryptose agar for the preparation of three pour plates, and (c) 8 c.c. of blood was placed into each of two flasks of 120 c.c. tryptose phosphate broth. The three types of cultures on each patient were incubated at 37° C. for 7 days.

The blood cultures were observed for growth after 24 hours, 48 hours, and 7 days of incubation. If no growth was observed after 7 days, the cultures were discarded as negatives. If growth was observed, the cultures were streaked on tryptose blood agar plates and pure cultures were prepared from each positive culture. From these, identification of the organisms were made and the antibiotic sensitivity studies performed.

Test Method for Determining Sensitivity of Bacteria to Antibiotics.—Although Terramycin was the antibiotic used in premedication, a comparison of each organism's sensitivity to other antibiotics was evaluated by the paper disk method. Those used in the sensitivity tests were Aureomycin, Bacitracin, Chloromycetin, dihydrostreptomycin, penicillin, and Terramycin. The tests were performed as follows: Six-inch diameter Petri plates of Bacto-Heart Infusion Agar containing 5 per cent human blood were prepared. These were inoculated by surface streaking with a suspension of the test organism so that a confluent growth would result. Excessive moisture was avoided. Immediately following the inoculation of the medium, Bacto-Sensitivity Disks* containing the antibiotics were placed on the surface, spaced about 3 or 4 cm. apart. The plates were incubated at 37° C. for 12 hours or until growth developed and observations for zones of inhibition were made.

*Supplied by Difco Laboratories, Inc., Detroit, Mich.

The 3 concentrations of each antibiotic supplied in the Difco antibiotic testing sets were used so that a more precise measure of the sensitivity to a given antibiotic could be obseryed. The antibiotic disks were arranged on the agar in the form of the spokes of a wheel. Each antibiotic formed a single spoke with the 3 concentrations aligned so that the least concentrated antibiotic disk would be the closest to the hub, and the greatest concentration at the distal end. A zone of inhibition of at least 1 mm. around the disk was interpreted as sensitivity to that antibiotic concentration. Resistant organisms were not inhibited by the maximum concentration of the antibiotic while very sensitive strains showed zones of inhibition by all the concentrations.

Oral Surgery Method.—The extractions were performed by the usual procedures of the Department of Oral Surgery as determined by patient requirements. After the routine aseptic technics of the clinic were carried out, the patient was administered a local anesthetic containing 1:50,000 Adrenalin as a vasoconstrictor.

The surgical procedure consisted of relieving the gingival attachment around the teeth to be extracted, loosening or elevating with the elevators, and extracting by a rotating motion of the anterior teeth or a buccal-lingual motion of the posterior teeth. The surgical site was closed with sutures or covered with a sterile gauze pack, depending on the number of teeth removed and the situation in each patient.

The Treatment of the Control Series.—The first 51 patients meeting the requirements for selection were handled in the routine manner. This group received no antibiotics before or after surgery. The blood cultures were collected as the surgeon finished his procedure.

Premedication Method for the Test Series.—The next 49 patients were treated in the same manner as the control series except for premedication. Each of these patients was given two 250 mg. capsules of Terramycin by mouth with 1 cup of water approximately 3 hours before oral surgery.

RESULTS

The control series had a total of 51 blood samples taken from the 51 untreated patients from whom one or more teeth had been extracted. Of these, 27 blood samples yielded one or more species of bacteria. The results of the control and premedicated groups are presented in Table I along with the types of bacteria and frequency of each.

A comparison of 20 (39.21 per cent) positive cultures among the control cases to 7 (14.28 per cent) positive cultures in the premedicated cases shows a significant reduction of bacteremia following premedication with Terramycin. It is of interest to note that *Streptococcus viridans* was the organism most frequently encountered. In only one control case was the blood positive with other bacteria, and in this patient *Staphylococcus aureus* and *Gaffkya* were found.

TABLE I

INCIDENCE OF DETECTABLE BACTEREMIA FOLLOWING ORAL SURGERY IN CONTROL AND TERRAMYCIN PREMEDICATED CASES

	NUMBER OF PATIENTS	POSITIVE CULTURE	PER CENT POSITIVE	STREPTOCOCCUS VIRIDANS	STAPHYLOCOCCUS AUREUS	NEISSERIA	GAFFKYA
Control series	51	20	39.21	19	5	5	1
Premedicated series	49	7	14.28	7	3	0	0
Totals	100	27		26	8	5	1

The presence of more than one species of bacteria in the blood cultures was not an unusual finding. In 4 cases in the control series, *Streptococcus viridans* was found in association with *Staphylococcus aureus* and in 5 cases *Streptococcus viridans* was found in association with *Neisseria*. On the other hand, the premedicated series not only showed fewer positive cultures, but the *Neisseria* and *Gaffkya* did not occur in this group. In 3 cases of the 7, *Streptococcus viridans* was found in association with *Staphylococcus aureus*.

If the data are examined from the viewpoint of the number of teeth extracted, as compared to the incidence of positive cultures in both series (Table II), it can be seen that single extractions showed a lower incidence of bacteremias; and as the number of teeth extracted increases, the incidence of positive blood cultures rises in both groups of patients. The significance of these data was tested by means of the chi square formula appropriate for a 2×2 table:

$$\chi^2 = \frac{N(ad - bc)^2}{(a + b)\ (c + d)\ (a + c)\ (b + d)}$$

where a, b, c, and d are observed frequencies in the 2×2 table and N is the total number of cases observed:

	+	−		
Control	a	b	$a + b$	df = 1
Premedicated	c	d	$c + d$	
	$a + c$	$b + d$	Total N	

	Blood Cultures +	−		% Positive
Control	20	31	51	39.2
Premedicated	7	42	49	14.2
	27	73	100	25.0% difference

$$\chi^2 = \frac{100\ (20{\cdot}42 - 31{\cdot}7)^2}{49{\cdot}51{\cdot}27{\cdot}73} = 7.88.$$

This demonstration of $\chi^2 = 7.88$ with P(probability) = 0.01 indicates a significantly smaller percentage of positive cultures in patients given the Terramycin as a premedication.

TABLE II

THE RELATIONSHIP OF NUMBERS OF TEETH EXTRACTED TO THE INCIDENCE OF POSITIVE BLOOD CULTURES

	TEETH EXTRACTED	POSITIVE BLOOD CULTURES	NEGATIVE BLOOD CULTURES	TOTAL CASES
Control series	1	1	6	7
	2	10	15	25
	3	1	6	7
	4	2	1	3
	5	3	2	5
	6	3	1	4
	Totals	20	31	51
Premedicated series	1	0	17	17
	2	1	12	13
	3	3	10	13
	4	0	1	1
	5	0	0	0
	6	2	0	2
	7	0	1	1
	8	1	1	2
	Totals	7	42	49

Table II reveals that positive cultures tend to be more common in patients having greater numbers of teeth extracted, both in the control and the premedicated groups. The numbers of cases having 1 through 8 teeth extracted are too small to permit exact treatment in 2×8 tables. Therefore, the data were condensed into 2 classes, those having N (the number of teeth extracted) equal to or greater than 4, and N equal to or less than 3. This gives, for the data on controls and premedicated groups combined, the following 2×2 table:

		Blood Cultures +	Blood Cultures −		% Positive
No. teeth extracted	$N \geq 4$	11	7	18	61.1
	$N \leq 3$	16	66	82	19.5
		27	73	100	41.6% difference

$$\chi^2 = 12.959,\ P < 0.001$$

Therefore, there is even stronger evidence favoring the assumption that the probability of a positive culture increases with the number of teeth extracted.

Thus, 2 factors have been shown to be significant in altering the likelihood of positive cultures: (1) the number of teeth extracted, and (2) the use of antibiotic premedication. This then poses the question: could the observed difference attributed to premedication be due to chance inequalities in the numbers of teeth extracted in the 2 series? Two facts may be adduced to the contrary. First, the mean number of teeth extracted (Table II) in the control patients is 139/51 or 2.7 per patient, only a trifle greater than that for the pre-

medicated group, namely, 121/49 = 2.5 per patient. Furthermore, upon classifying patients into 2 groups having 4 or more teeth extracted ($N \geq 4$) and 3 or less extracted ($N \leq 3$), we find the following 2×2 tables:

$N \geq 4$ Teeth	+	−	Tot.	% Positive
Controls	8	4	12	66.7
Premedicated	3	3	6	50.0
	11	7	18	16.7% difference

$\chi^2 = 0.468$
$P = 0.5$

$N \leq 3$ Teeth	+	−	Tot.	% Positive
Controls	12	27	39	30.7
Premedicated	4	39	43	9.3
	16	66	82	21.4% difference

$\chi^2 = 6.001$
$P = 0.02$

In the larger group, with $N \leq 3$, the excess proportion of positive cultures in controls is again significant at the $P = 0.02$ level; the deviation in the $N \geq 4$ group, although in the same direction, is not significant. We may show further that the difference in percentage of positive cultures (controls minus premedication), which is 21.4 per cent in the $N \leq 3$ group, is not significantly different from that in the $N \geq 4$ group, namely, 16.7 per cent. This is done by computing the "interaction" chi square for a 2×2 table (see Snedecor, p. 202).[13] We first solve the cubic equation

$$(12 - x)\ (39 - x)\ (3 - x)\ (4 - x) = (4 + x)\ (27 + x)\ (3 + x)\ (8 + x)$$

where the numbers 12, 39, etc., are taken from the 2 tables above. The solution is the real root $x = 0.5348$. Chi square is now computed as

$$\chi^2 = x^2\left(\frac{1}{12 - x} + \frac{1}{39 - x} + \frac{1}{3 - x} + \frac{1}{4 - x} + \frac{1}{4 + x} + \frac{1}{27 + x} + \frac{1}{3 + x} + \frac{1}{8 + x}\right) = 0.389$$

$P > .50$

The nonsignificance of the result $\chi^2 = 0.389$ indicates that although the number of cases having 4 or more teeth removed is small, the results for premedicated and untreated cases are in line with those found in the group in which 3 or fewer teeth were removed.

The comparative study of all isolated organisms as to their in vitro sensitivity to antibiotics revealed the data presented in Table III. In the case of *Streptococcus viridans,* all 26 strains isolated were sensitive to Terramycin, Chloromycetin, Aureomycin, and Bacitracin. Two of these were resistant to penicillin, and 5 strains were resistant to dihydrostreptomycin. In addition, 11 strains were only slightly sensitive to penicillin, showing zones of inhibition with the impregnated disk having 10 units but not with those disks having 0.1 unit and 1.0 unit. Eleven of the 21 strains isolated were only slightly sensitive to dihydrostreptomycin, showing a zone of inhibition at 100 mg., but not at 10 or 1 mg.

All strains of *Staphylococcus aureus* were sensitive to Chloromycetin, Aureomycin, and Terramycin, 3 were resistant to penicillin, 1 was resistant to dihydrostreptomycin, and 3 were resistant to Bacitracin. The isolated strains of *Gaffkya* and *Neisseria* were sensitive to all of the antibiotics tested.

TABLE III

ANTIBIOTIC TESTS ON THE ORGANISMS ISOLATED FROM PATIENTS FOLLOWING ORAL SURGERY TABULATED AS THOSE SENSITIVE

ORGANISM	NUMBER ISOLATED	PENICILLIN	DIHYDROSTREPTOMYCIN	CHLOROMYCETIN	AUREOMYCIN	BACITRACIN	TERRAMYCIN
Control Series							
Streptococcus viridans	19	17	16	19	19	19	19
Staphylococcus aureus	5	2	4	5	5	5	5
Neisseria	5	5	5	5	5	5	5
Gaffkya	1	1	1	1	1	1	1
Premedicated Series							
Streptococcus viridans	7	7	5	7	7	7	7
Staphylococcus aureus	3	3	3	3	3	0	3

DISCUSSION

In a study of this nature the presence of infection in and around the extracted teeth must be considered as a factor which may influence the data. Table IV shows the separation of the cases into 2 categories on the basis of clinical and radiologic diagnosis; those having obvious infection and those apparently showing no infection. Those cases classified as infected showed periodontal disease, pulp exposure, pericoronal infection, or periapical pathosis. The noninfected teeth were extracted because of caries beyond restoration, impaction, malposition, or malocclusion.

TABLE IV

INCIDENCE OF CLINICALLY DIAGNOSED INFECTED TEETH AND SURROUNDING STRUCTURES IN CONTROL CASES AS COMPARED TO PREMEDICATED CASES

	INFECTED TEETH OR SURROUNDING STRUCTURE	NONINFECTED TEETH OR SURROUNDING STRUCTURE	
	Control		
Positive cultures	11	9	
Negative cultures	21	10	Total = 51
	Premedicated		
Positive cultures	7	0	
Negative cultures	31	11	Total = 49

Table IV shows that of the 20 bacteremias found in the control series, 9 were from noninfected cases as compared to 11 positive cultures from the infected cases. In the premedicated series all 7 of the bacteremias were secured from patients who were definitely in the group showing clinical evidence of infection. This would indicate that Terramycin as used acted as a

good premedication except where grossly infected teeth or surrounding tissues were involved. It should also be noted that in the premedicated series there were only 7 (18.4 per cent) positive blood cultures from a total of 38 cases of obvious infection as compared to 11 (34.4 per cent) positive cultures obtained from a total of 32 infected patients. The incidence of positive cultures from infected patients in the control series is greater than in the premedicated series. This becomes more evident when those cases failing to show clinical or radiologic infection are examined. The 19 "noninfected" cases of the controls yielded 9 (47.4 per cent) positive blood cultures, while none of the 11 "noninfected" cases of the premedicated series showed positive bacteremia.

The number of teeth extracted in any one surgical procedure had a significant effect upon the incidence of bacteremia in this study. The greater the number of teeth extracted, the more likelihood of bacteremia. This relationship of the extent of oral surgery to the incidence of bacteremia is the expected when one considers that the removal of each tooth results in additional exposed alveolar spaces, more venous canals ruptured, and more trauma.

It is of interest to find evidence that agents other than antibiotics may influence the occurrence of bacteremia in patients undergoing oral surgery. Okell and Elliott found that 60.9 per cent of their cases showed bacteremia. Their study was made using general anesthesia. In contrast to this, Burket and Burn found that only 16.9 per cent of their patients showed a bacteremia when a local anesthetic containing 1:20,000 Adrenalin was used. The local anesthetic used in the present report contained 1:50,000 Adrenalin and our results in the control patients showed a frequency of 39.21 per cent bacteremias. If one can assume that the methods of the 3 reports are comparable, the incidences of bacteremias show a relationship to the amount of vasoconstrictor used with the anesthetic.

Although such a comparison between the 3 sets of data might lead to the conclusion that increased amounts of Adrenalin could be a means of reducing bacteremia, other important considerations enter into such an evaluation. Excessive Adrenalin might lead to severe reduction of blood supply to the area with undesirable results. This raises possible objections to the use of high concentrations of vasoconstrictors in oral surgery for the primary purpose of preventing bacteremia.

Our results show a significant reduction of bacteremia in the premedicated patients who received a single 500 mg. oral dose of Terramycin as compared to the untreated controls. The 7 bacteremias of the premedicated series were obtained from patients who had gross infections in the oral cavity. One might assume from this that a more vigorous prophylactic course of antibiotics administered before surgery could have reduced the incidence of bacteremia in such patients.

An effort was made to observe the premedicated patients postoperatively. The surgical sites were examined to see if there was any noticeable change in the normal healing process due to the premedication. In no case was there a

recognizable interference with normal tissue healing, and in a few cases there seemed to be slight acceleration. None of the patients in the control or premedicated series developed alveolitis or other postoperative complications due to infection.

SUMMARY

When blood cultures are made on patients immediately following oral surgery, a high incidence of bacteremia can be demonstrated. When such individuals are given adequate doses of antibiotics, such as Terramycin, the incidence of demonstrable bacteria in the blood stream is markedly reduced. This reduction is more evident in the group having no clinical signs of infected teeth or surrounding tissues.

Positive blood cultures were higher when a greater number of teeth was removed at any one time. This incidence of bacteremia was markedly reduced in the premedicated patients by the administration of oral Terramycin 3 hours preoperatively.

The organism most commonly found was *Streptococcus viridans*. In all cases the strains of *Streptococcus viridans* isolated showed an in vitro sensitivity to the antibiotic used in this report.

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THE RELATION OF SYSTEMIC FLUORIDE AND THYROID GLAND ACTIVITY TO THE INCIDENCE OF DENTAL CARIES IN THE RAT

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PREVIOUS work has shown a relationship between the activity of the thyroid gland and the incidence of experimental dental caries in the rat. For example, feeding desiccated thyroid significantly reduces the dental caries incidence, whereas thiouracil significantly increases the dental caries incidence.[1] Destruction of the thyroid gland with radioactive iodine produces the same cariogenic effect as feeding thiouracil.[2] Since Muhler and Shafer[1] have presented data which indicate that thyroid feeding and fluorine supplements furnished in the drinking water of rats (thus administered topically) may be synergistic in their anticariogenic actions, the purpose of this paper was to test the effect of fluoride injected intraperitoneally upon the reduction in dental caries produced by feeding desiccated thyroid.

EXPERIMENTAL

A total of 75 weanling male albino rats (Sprague-Dawley strain) were equally divided as to initial body weight into 3 experimental groups. Group 1 received desiccated thyroid in the diet (in increasing amounts from 5 to 15 mg. per day depending upon body weight gain) while Group 2 received the same amount of desiccated thyroid in the diet and in addition were injected intraperitoneally each day with 0.3 mg. of fluorine (as NaF). This amount of fluorine was selected since it is approximately the same as the daily intake of animals receiving drinking water containing 20 μg per milliliter of fluorine, a level known to produce a reduction in dental caries in the rat.[3-5] Group 3 received no supplements and served as a control. All animals were fed a coarse corn caries-producing diet* and distilled water for 120 days. At the termination of the experiment the animals were sacrificed by ether and the heads were removed and examined for dental caries by methods previously described.[6]

DATA AND DISCUSSION

The anticariogenic effect of feeding desiccated thyroid is shown in Table I and confirms the previously published work on this subject.[1, 2, 7] Desiccated thyroid is not only effective in preventing the initiation of the carious lesions in rats as evidenced by the reduced number of carious teeth (molars affected),

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*The composition of the diet used in this study was as follows: yellow corn grits, 52.7%; ground yellow corn, 11.3%; powdered whole milk, 30.0%; alfalfa, 4%; iodized sodium chloride, 1.0%, and irradiated yeast, 0.2%.

but is equally effective in reducing the mean number of lesions ($p = 0.01$) and the severity of the cavity. Table I also indicates that the injection of 300 μg of fluorine per day (as NaF), into animals which were fed desiccated thyroid, results in essentially the same anticariogenic effect as feeding thyroid alone. No significant differences were observed in the number of molars affected, the severity of the lesions or the mean number of lesions in the 2 groups. Keyes and Shourie[8] have indicated that NaF injection may reduce caries in the hamster. They emphasize, however, the possible variable in their experiments of topically applied fluorine from the feces since these animals practice coprophagy. Zipkin and McClure[9] have found that 500 μg per day of injected fluorine from several different fluoride compounds is ineffective in reducing dental decay in the rat. These studies seem to indicate that systemic fluoride administered posteruptively is ineffective in reducing dental caries, and the results reported here tend to confirm those data.

TABLE I

THE EFFECT OF THYROID FEEDING AND FLUORIDE INJECTION ON THE INCIDENCE OF DENTAL CARIES IN THE RAT

GROUP	NUMBER OF ANIMALS	FINAL WT. OF ANIMALS (GM.)	MOLARS AFFECTED	MEAN NUMBER OF LESIONS	PERCENTAGE DIFFERENCE	SEVERITY
Control	21	325	4.0	7.3 ± 2.5†	—	2.56‡
Desiccated thyroid	21	298	2.8	4.4 ± 1.8	-36.3	1.99
Desiccated thyroid plus 300 μg fluorine/day*	19	295	2.8	4.2 ± 2.4	-39.1	2.03

*Intraperitoneal injection.

†Standard deviation.

‡Computed by estimating the degree of damage to molar teeth. If from zero to one-fourth of the tooth is destroyed, a value of one is assigned to this tooth; if one-fourth to one-half is decayed, a value of two is indicated, while if more than one-half is carious, the tooth is given a value of three. The arithmetic mean is then calculated for each group.

Previous work[1] had indicated a possible synergistic anticariogenic effect of thyroid hormone and fluoride which was not explained. The data presented here emphasize the inefficacy of systemic fluoride in the prevention of dental caries in erupted teeth and point out that topical application of fluoride to posteruptive teeth is most likely the modus operandi in the anticariogenic action of fluorides in both untreated and thyroid-fed rats.

SUMMARY

The anticariogenic effect of feeding desiccated thyroid to rats was again confirmed in this study. The synergistic anticariogenic effect of fluoride administered in the drinking water with the effect of feeding desiccated thyroid has been shown to be the result of topical fluoride treatment since the synergistic action of fluoride is not apparent when injected intraperitoneally in rats receiving desiccated thyroid.

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LABORATORY METHODS FOR SCREENING POSSIBLE INHIBITORS OF DENTAL CARIES

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IT is generally believed by dental research workers that acids formed by oral bacteria acting on fermentable carbohydrates play an important role in the production of dental caries.[1] Various workers have pointed out that the dento-bacterial plaque, which is normally present on tooth surfaces, has the ability to produce acid when incubated with glucose solutions[2, 3] or when fermentable carbohydrates are taken into the oral cavity.[4, 5] It has also been noted that caries is likely to occur at locations on the teeth which are normally covered with plaque[6, 7] and relatively inaccessible to either natural cleansing or cleansing with a toothbrush. Since plaque has the ability to form acid and is attached firmly to various surfaces of the teeth, it seems reasonable to assume that it is intimately connected with the carious process.

Investigations indicate that the plaque is primarily protein in nature and is composed, at least in part, of living and dead microorganisms and denatured salivary protein.[8-12] If the plaque were treated in some manner so that its ability to produce acid was reduced then, according to theory, a reduction in tooth decay might occur. It has been reported in the literature that dental plaque can be modified by the addition of certain materials so that its acid-forming action, as determined by pH readings of the plaque in the oral cavity, is materially reduced.[13, 14] However, the use of a plaque pH method as a screening procedure to test a large number of compounds would be extremely time consuming and toxicity data would be a necessary prerequisite to testing. Therefore a need existed for a rapid in vitro method for preliminary screening which would measure the ability of a substance to interfere with acid production as well as its ability to be retained in the mouth or to leave a residual effect for a reasonable length of time.

Various in vitro tests are known which can be applied to measure acid inhibition. The Snyder test[15, 16] measures acid production by the rate of change of color of an indicator, bromcresol green, in a special medium inoculated with saliva. A method reported by Wach, Kesel, Hine, and O'Donnell[17] evaluates acid production of saliva-glucose mixtures by pH readings and was somewhat modified in the present study. In these procedures, the use of caries-active saliva results in appreciable acid formation. Various materials may be added and if effective will reduce the amount of acid formed. These tests indicate whether or not a material is an acid inhibitor at low concentrations.

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Since plaque is composed, at least in part, of protein material, it was decided to determine if any retention of acid inhibitors occurred when they were used to treat proteinaceous materials. Several protein or protein-like materials were studied. It was found that certain caseins acted similar to precipitated salivary mucin in that they adsorbed some inhibitors in an active form, while other inhibitors were either not adsorbed or were inactivated by protein. Although it has not been determined that a true adsorption occurs, this appears to be the most likely mode of action. Therefore, one type of casein* was used in much of this work. Casein offers advantages in availability, uniformity, ease of handling, and weighing.

TEST METHODS

Protein Adsorption Test.—

a. *Casein adsorption test—Snyder medium:* One gram of casein is treated at room temperature for 1 hour with a 50 ml. aqueous solution (concentration usually 0.25 per cent) of the test material. At the end of this period, the solution is poured off and the protein washed by decantation 10 times with 50 ml. portions of distilled water. The protein is filtered, washed again with 50 ml. of water and dried in a vacuum desiccator at room temperature. Weighed portions of the treated casein (up to 300 mg.) are then added to tubes of liquefied bromcresol green dextrose agar to which 0.2 ml. of caries-active saliva has previously been added (temperature, 55° to 60° C.). The tubes are agitated vigorously, the casein allowed to settle and then incubated at 37° C. Control tubes containing agar, caries-active saliva and water-treated casein are also prepared. The latter tubes will have a predominantly yellow color at the end of 24 hours. If the test substance is picked up by the protein, retained during washing and is present in sufficient quantity in an active form, then the tubes with treated casein will remain green for 72 hours of incubation. In this test, it is possible to add varying amounts of treated casein to the tubes and thereby increase or decrease the amount of inhibitor as desired. It is also possible to obtain greater pick-up in many cases by treating the casein with solutions of a higher concentration, although with surface active materials an increase in concentration will sometimes cause solubilization of casein.

The compounds are classified according to their effectiveness in the following manner: those rated as "active compounds" are effective when 100 mg. or less of one gram of casein treated with an aqueous solution of no greater concentration than 0.25 per cent is added to the medium; those listed as "slightly active compounds" are effective only at concentrations greater than 0.25 per cent; a third group consists of compounds, some of which are mentioned in the literature as acid inhibitors, which were not effective in these tests under the conditions employed.

Saliva from different individuals will cause some variations in the results obtained, but it has been our experience that analogous results are obtained when saliva which is rated caries-active by the Snyder test is employed,

*"New Process" Casein (20-40 mesh), Sheffield Farms Company, Inc., Chemurgic Division, Norwich, N. Y.

even though obtained from different subjects. However, if the caries-active saliva used is collected from the same person then the results, in general, are reproducible.

b. *Casein adsorption test—glucose medium:* A similar test involves the addition of 100 mg. of casein treated as described in the Snyder medium test to 4 ml. of caries-active saliva and 1 ml. of a 20 per cent glucose solution. This mixture is incubated for 4 hours at 37° C. and the pH determined before and after incubation. A control containing untreated casein, pooled saliva, and glucose solution is also prepared. Attempts to vary the amount of casein in the saliva-glucose mixture result in difficulties since in this type of liquid mixture, casein has a definite buffering effect. Therefore 100 mg. of casein is used in all cases, with the concentration of the solution used to treat the casein being varied as desired.

Again the compounds are classified according to their effectiveness. Those rated as "active compounds" maintained the pH of the mixture 1.5 pH units above the control when 100 mg. of 1 gram of casein treated with an aqueous solution of no greater concentration that 2.5 per cent is added to the medium. Those listed as "slightly active compounds" were effective only at higher concentrations.

Salivary Protein Adsorption Test—Snyder Medium.—

A few selected inhibitors were also studied using salivary protein from pooled saliva. The salivary protein was isolated by titrating saliva to a pH of 2.6 and salting out the protein with sodium sulfate. It was then washed by dialysis and dried in a vacuum desiccator at room temperature. Although it was realized that this was a fairly extreme treatment, the use of salting-out procedures under less acid conditions resulted in material which was partially water-soluble and therefore not adequate for use in these tests which call for the treatment of an insoluble proteinaceous material with aqueous solutions.

This precipitated protein was then exposed to solutions of inhibitors as outlined under casein adsorption test—Snyder medium and added to Snyder agar inoculated with caries-active saliva.

The compounds are classified in the same manner as described above under the casein adsorption test—Snyder medium method.

RESULTS

The majority of the results reported here are based on the casein adsorption test in Snyder medium. This method was first applied to various antibiotics and it was found that penicillin-treated casein was effective in reducing acid production by saliva, while certain other antibiotics were not very effective in this casein test. At the time this work was in progress, a report indicated that penicillin was clinically effective in limiting dental caries.[22] Therefore, the test was further applied to other compounds to determine if they exhibited activity under these conditions similar to the activity obtained with penicillin.

Several hundred compounds were screened by adding them to Snyder medium; of these a fairly large percentage inhibited acid formation. However, as may be seen in Table I, only a few were effective after being used to treat casein.

TABLE I
CASEIN ADSORPTION TEST—SNYDER MEDIUM*

Active Compounds
Phenyl mercuri acetate
Na-p-hydroxy mercuri benzoate
O-chloromercuri phenol
Penicillin G (K salt)
Aureomycin HCl
Chloromycetin
Na N-lauroyl sarcosinate
Na N-myristoyl sarcosinate
Terramycin HCl
Na N-palmitoyl sarcosinate
Terramycin HCl
Na N-palmitoyl sarcosinate
5-nitro-2-furoic acid
Acridine derivative†
Slightly Active
Iodoacetic acid
Subtilin
No Activity Observed
Bacitracin
Cetyl trimethyl ammonium bromide
Neomycin
d-Panthenol
l-Phenyl-l-decyl cinnamyl dimethyl ammonium chloride
l-Phenyl dodecyl benzyl-dimethyl ammonium chloride
Sodium copper chlorophyllins
Sodium N-stearoyl sarcosinate
Sodium laurate
Sodium myristate
Sodium palmitate
Sodium lauryl sulfate
Sodium lauryl sulfoacetate
Sodium fluoride
Sodium dehydroacetate
Tyrothricin
Polymyxin B-sulfate
Sarcosine HCl
Sulfated monoglycerides of coconut oil fatty acids (sodium salt)
Glyceraldehyde
Disobutyl-cresoxy-ethoxy-ethyl-dimethyl benzyl ammonium chloride monohydrate
B-carboxy-N-dodecyl propionamide (sodium salt)

*Listed in order of activity.
†Obtained from Abbott Laboratories as 9-parahexyl oxy-phenyl-9-chloro-10-methyl acridine.

Concurrently with the screening program using casein as the protein material, a few selected inhibitors were also studied using salivary protein isolated from saliva. The results obtained are summarized in Table II.

When results from the casein adsorption test in Snyder medium are compared with results obtained using salivary proteins a general agreement is found, with the exception of 2 mercurials. It is believed that any material that is effective with casein should also be tested with salivary protein before it is considered for further investigation. This type of testing procedure was

TABLE II
SALIVARY PROTEIN ADSORPTION TESTS—SNYDER MEDIUM*

Active Compounds
Penicillin
Aureomycin HCl
Na N-lauroyl sarcosinate
Chloromycetin
Terramycin HCl
5-nitro-2-furoic acid
Na N-myristoyl sarcosinate
Slightly Active
Sodium N-palmitoyl sarcosinate
No Activity Observed
Cetyl trimethyl ammonium bromide
Sodium N-stearoyl sarcosinate
Phenyl mercuric acetate
Sodium p-hydroxy mercuri benzoate

*Listed in order of activity.

subsequently developed by others.[18-21] Fosdick and co-workers built up artificial plaque on extracted teeth and then treated this plaque with various antibiotics and other materials. These investigators found penicillin, Aureomycin, Terramycin, subtilin, some amino acid derivatives of 5-nitro furoic acid, sodium N-lauroyl sarcosinate, sodium dehydroacetate, sodium N-myristoyl sarcosinate, 1-phenyl-1-decyl-cinnamyl-dimethyl-ammonium chloride and 1-phenyl dodecylbenzyl-dimethyl-ammonium chloride were effectively retained on the plaque by their method. When these compounds were evaluated in the casein adsorption test using the Snyder medium they were all effective with the exception of sodium dehydroacetate and the 2 quaternary ammonium salts. It is interesting to note that in the method described in this paper the tubes are evaluated after 72 hours of incubation, while in the test employed by Fosdick and co-workers the tubes are read after 48 hours of incubation, a difference of 24 hours.

Certain substances were also studied in saliva-glucose mixtures (casein adsorption test—glucose medium). It may be noted that this medium differs from the Snyder medium in that it calls for a liquid with an excess of saliva, rather than a gel. The data are summarized in Table III.

TABLE III
CASEIN ADSORPTION TEST—GLUCOSE MEDIUM*

Active Compounds
Phenyl mercuri acetate
O-chloromercuri phenol
Penicillin G (K salt)
Na-p-hydroxy mercuri benzoate
Aureomycin HCl
Acridine derivative
5-nitro-2-furoic acid
Chloromycetin
Sodium N-lauroyl sarcosinate
Slightly Active
Sodium N-palmitoyl sarcosinate
Terramycin HCl
No Activity Observed
Cetyl trimethyl ammonium bromide

*Listed in order of activity.

Consideration of materials that are effectively retained by casein indicates that some antibiotics, notably penicillin, Aureomycin hydrochloride and Chloromycetin are active at low concentrations. Reports concerning the effectiveness of penicillin dentifrices in reducing dental caries are conflicting.[22-25, 27] However, the one favorable clinical report by Zander was a regimented study using a powder containing 500 units of potassium penicillin G. This material is quite effectively adsorbed by both casein and salivary protein. However, the use of antibiotics may not be justified in dentifrices, in view of their more important applications in other fields and the potential danger of development of resistant strains of bacteria.

Certain mercurials also show rather marked effectiveness when adsorbed by casein, but this was not confirmed when salivary protein was substituted for casein. Furthermore, although sodium-p-hydroxy mercuri benzoate in low concentrations has been proposed as a caries-preventive agent,[26] mercurials are rather toxic substances for oral use.

Although some quaternary ammonium compounds were effective in preventing acid formation at very low concentrations when used directly they were completely inactive after being adsorbed on casein. Preliminary work in our laboratories indicate that they are picked up by casein but are bound too tightly to the surface of the casein to be effective.

A series of sodium salts of fatty acids were quite effective in direct addition tests, but were inactive in the protein adsorption tests. On the other hand, sodium salts of the condensation products of sarcosine with various fatty acids including the lauroyl, myristoyl, and palmitoyl derivatives showed varying degrees of activity after being adsorbed on casein. A similar result was found when precipitated salivary protein was employed. Since these compounds are detergents, other detergents were studied, such as sodium lauryl sulfate, sodium lauryl sulfoacetate and sodium coconut monoglyceride sulfate. None of these were effective in the protein adsorption tests.

It is believed that the first step in screening materials should involve the determination of their effectiveness as acid inhibitors by direct addition to either inoculated Snyder test agar or saliva-glucose mixtures. If the material inhibits acid production at a reasonably low concentration, then further testing to determine retention on casein and salivary protein and inhibition of acid formation, while so retained, is indicated. Assuming that dental caries results from acid formed by oral bacteria or their enzyme systems acting on fermentable carbohydrates and that this acid is held in close contact with the tooth surface by dental plaque, then the ability of a compound to be picked up and held by proteinaceous material and to reduce acid production by salivary bacteria could indicate a possible dental caries inhibitor. The methods presented offer quick convenient testing of large numbers of materials in the laboratory. They are not offered as substitutes for animal experiments or clinical testing, but merely as preliminary screening devices to indicate potential caries inhibitors worthy of further study.

SUMMARY

The Snyder and Wach tests for caries activity have been modified and adapted to the screening of materials as possible inhibitors of salivary acid

formation. To be an effective anticaries agent it is believed that such an inhibitor should be retained in the mouth, on the dental plaque or oral tissue, and exercise a continuing restraint on acid production.

Precipitated salivary mucin and a commercial casein have been found to retain certain inhibitors in active form. When the treated proteins are introduced into an appropriate nutrient with acidogenic salivary organisms they reduce acid formation.

Screening results by these tests on several dozen compounds are given. Among the more effective inhibitors by these tests are penicillin, Aureomycin hydrochloride, Chloromycetin, sodium N-lauroyl sarcosinate, Terramycin hydrochloride, and 5-nitro-2-furoic acid.

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THE ACTION OF INDIVIDUAL BACTERIAL STRAINS ON HUMAN TEETH UNDER IN VITRO CONDITIONS

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IT is accepted generally that dental caries involves the action of microorganisms and the products which they produce. Much research has been done with the purpose of establishing the nature of the microorganisms involved in the carious process. However, no general agreement has been reached. The concept that various oral microorganisms may participate in the carious process was accepted widely until about 1920.

During the succeeding years, numerous attempts were made to involve a specific organism in the carious process. Several organisms were implicated, but the search resulted in the concept that lactobacilli species were the specific organisms.[17, 35] Much of the evidence for this concept was based on the occurrence of lactobacilli in lesions and on correlations which were observed between lactobacillus counts, caries indices, and amount of dietary carbohydrates. However, as pointed out by Jay,[17] "Many do not agree that causal relationships exist between the lactobacilli and dental caries. Regardless of this controversial point, these organisms afford an excellent means of estimating caries activity." Thus, as implied by Jay,[17] lactobacilli could be organisms which indicate carious conditions without necessarily being the principal etiological agent.

Although the concept of lactobacilli species as specific agents of caries has become fairly popular, much evidence has accumulated to show that other organisms are involved and that they may be of greater importance than lactobacilli. Thus, Anderson and Rettger,[1] and Bibby, Volker, and Van Kesteren[2] showed that numerous streptococci species were even more acidogenic than lactobacilli, occur in much greater numbers in the mouth, and probably are of more significance than the lactobacilli. After an examination of the evidence, Cox[6] essentially reiterated in modern terms the earlier conclusions of Miller[21] and finally concluded that no specific bacterial species is responsible for caries. These conclusions received still further support in the results obtained by the inoculation of "germ free" animals with specific organisms other than lactobacilli.[24]

Further evidence has been provided by a number of studies of the in vitro production of carious lesions by the action of individual strains of microorganisms.[1, 3, 5, 7, 11, 13-16, 19, 20, 36] Single oral strains of lactobacilli, streptococci, micrococci, and other bacteria were reported as cariogenic agents.

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The artificial mouth is a development of in vitro procedures which probably provides a greater control of variables and a closer correspondence with oral conditions than previously was possible. The conditions under which lesions are produced and the progress of the lesions appear to be very similar to those produced in the human mouth.[30] By a slight modification of the original apparatus, it is possible to study the effect of single strains of microorganisms. Because of the lack of general agreement on the nature of the organisms responsible for the carious process, the artificial mouth has been used in the present work to study the action of specific bacteria on the different tissues of teeth. The present paper reports the results that have been obtained in a study of the action of nine strains of oral microorganisms. Preliminary reports of this investigation have been given elsewhere.[10, 29, 31]

EXPERIMENTAL APPARATUS AND METHOD

The apparatus and method used previously[27] were adapted for the use of pure strains of microorganisms. The general method consisted of mounting an extracted human tooth in dental stone in an acrylic box. The mounted tooth was placed in a cylindrical funnel, and a nutrient solution was allowed to drip on the tooth continuously for the entire experimental period of 25 weeks. During this period, a known microorganism was maintained on the tooth surface.

The modified apparatus is shown in Fig. 1. A reservoir containing the bacteriologic medium was connected to a drip tube which in turn was attached to a piece of glass tubing inserted through a No. 10 rubber stopper. In order to protect the upper surface of the cylindrical funnel from bacterial contamination, an angular groove had been cut in the lower surface of the rubber stopper; this groove was about ¼ inch deep and had the same diameter as the cylindrical funnel (Fig. 1). The mounted tooth was placed in the cylindrical funnel which was closed at the top with the rubber stopper. The bottom of the funnel was connected to another drip tube which in turn was attached to a piece of glass tubing about 12 cm. in length. This tubing was long enough to reach within ¼ inch of the bottom of the bottle which received the waste medium. This exit tube was always kept covered with water saturated with sodium fluoride and mercuric chloride. The flow of the media over the tooth was regulated at about 2 to 4 drops per minute by the use of a pinchcock above the upper drip tube. With this rate of flow, about 200 to 400 c.c. of media was used every 24 hours. The apparatus was placed in a constant temperature cabinet maintained at 35° to 36° C.

At the start of the experiment, the entire apparatus with the exception of the bottle of bacteriologic medium was assembled and autoclaved at 120° C. for 20 minutes. The medium, in a 2-liter bottle which carried a short length of rubber tubing at the outlet, was autoclaved separately for 40 minutes at 120° C. Fresh medium could thus be connected to the apparatus whenever needed.

The cylindrical funnel (Fig. 1) carried a side arm through which inoculations could be made. The side arm was closed with a cotton plug and covered with an inverted test tube. Prior to the introduction of bacteria, the exit

tube was closed with a pinchcock, and the medium was allowed to accumulate in the cylindrical tube until it reached the height of the tooth. By aseptic procedures, 1 to 2 c.c. of a bacterial suspension was introduced by means of a Pasteur pipette. The bacteria were allowed to grow in this medium for a period of 24 hours before the regular continuous dripping was commenced.

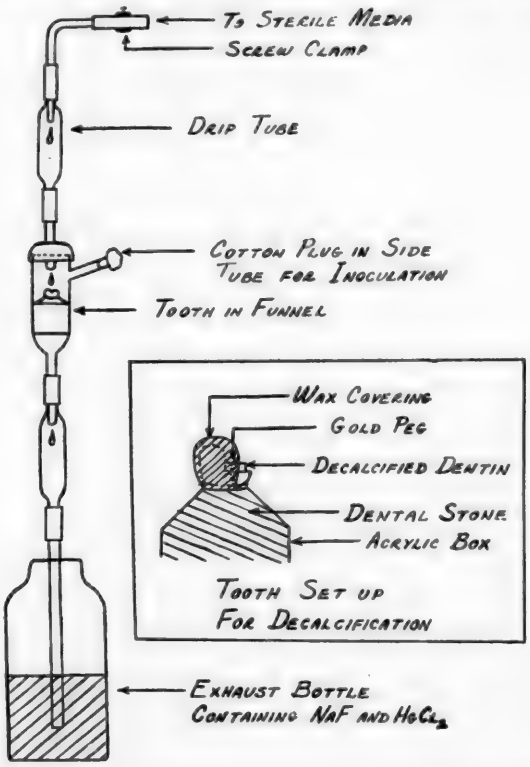

Fig. 1.—Apparatus for artificial mouth for experiments with single strains of microorganisms.

At weekly intervals, a small sample of the microorganisms growing on the tooth surface was removed by means of a platinum loop inserted through the side arm. This sample was tested for the possible presence of contaminating organisms. The tooth surfaces were examined visually with a low-power microscope and recorded by photographs taken initially and after a period of 8 and 25 weeks.

Preparation and Mounting of Teeth.—The teeth were third molars extracted from individuals whose ages fell in the range 15 to 43 years. None of the teeth had fully erupted, and none showed any signs of caries or decalcification. Immediately after extraction, the teeth were cleaned, placed in isotonic saline and kept at 2° to 3° C. until needed.

In order to study the differences in susceptibility of the various tooth fractions, the crowns were cut so as to expose the intact surface enamel, the

cut enamel, and the cut sound dentin (see Fig. 1). In addition, a portion of the sound dentin was decalcified with acids in order that decalcified dentinal matrix would be present. A portion of the original cementum was also left exposed on each tooth.

This was accomplished by mounting the intact tooth in dental stone high enough so that a millimeter of cementum was exposed. The surface of the dental stone was covered with cold-setting acrylic resin which was brought into contact with the tooth slightly below the cemento-enamel junction. One quarter of the crown was removed by the use of a dental disc with one cut made axially from the buccal to the lingual surface and the other made horizontally 4 to 5 mm. below the cusp tips. A small hole was drilled vertically in the surface of the cut dentin, and a piece of gold wire (0.022 orthodontic wire) was cemented with zinc phosphate dental cement into the hole; the upper end was flush with the cut dentin. The entire tooth was imbedded in wax, which was cut away so as to expose about 2 to 3 mm. of the cut dentin. The exposed section of the dentin and some of the enamel below it were subjected to a continuous flow of 0.001 *N* HCl for several weeks. This portion of the dentin was then found to be spongy and highly decalcified. The wax was then removed mechanically and finally by the use of ether as a solvent. The initial condition of 4 of the teeth is shown in Fig. 2.

Bacteriologic Medium.—The medium used was that described previously.[32] It was composed of 0.25 per cent D-glucose, 0.5 per cent Bactotryptone, 0.2 per cent yeast extract, and 0.025 per cent $CaHPO_4 \cdot 2H_2O$ at pH 6.2. The medium was made up and sterilized in 2 liter bottles. As a bottle became empty, it was removed and replaced aseptically by a bottle of fresh medium.

Sources and Types of Microorganisms.—The microorganisms used for the present work were as follows: *Lactobacillus casei* (ATCC 4646, Hadley strain); *Lactobacillus casei* (isolated from saliva at Tufts College Dental School by Mrs. Inga Mahler); *Streptococcus faecalis* (ATCC 4083, Hadley strain); *Streptococcus salivarius* (ATCC 9756, Niven strain); *Streptococcus salivarius* (isolated from saliva at the University of Alabama Dental School by Miss C. Gaston and Frederick W. Kraus); *Micrococcus pyogenes* var. *aureus* (hemolytic and coagulase-positive, isolated from saliva at the University of Alabama Dental School by Frederick W. Kraus); *Micrococcus pyogenes* var. *albus* (nonhemolytic and coagulase-negative, isolated from saliva at the University of Alabama Dental School by Frederick W. Kraus); *Clostridium perfringens* (ATCC 846, Torrey strain); *Neisseria catarrhalis* (isolated from saliva at Tufts College Dental School by Mrs. Inga Mahler).

The following organisms were not cultivated successfully under the conditions previously described for the artificial mouth: *Lactobacillus fermenti* (provided by W. W. Carlson, origin unknown); *Streptococcus mitis* (isolated from saliva at Tufts College Dental School by Mrs. Inga Mahler, No. 23); *Corynebacterium hoffmannii* (isolated from saliva at Tufts College Dental School by Mrs. Inga Mahler).

The organisms were maintained by regular transfers to suitable media before use. Except for lactobacilli and *Clostridium perfringens*, the inoculum was prepared by culturing the organisms for 12 and 24 hours in Difco Brain-Heart Infusion broth at pH 7.4. For the lactobacilli strains, the same broth was used except that the pH was adjusted to 5.6. *Clostridium perfringens* was grown similarly in Difco Thioglycollate broth. Each tooth was inoculated with 1 to 2 c.c. of a 12 to 24 hour culture.

Weekly examinations of the purity of the organisms on the teeth were made. This was done by removing a small sample from the surface of a tooth by a platinum loop, and transferring to Thioglycollate broth and Brain-Heart Infusion broth. Aerobic and anaerobic contamination was checked by this procedure. The organisms which grew in the media after 24 hours were streaked on blood agar plates, and the colonies examined after 48 hours. Occasionally, contamination was observed. In every instance but one, the contaminating organism was *Bacillus subtilis*. The single exception was a micrococcus.

When contamination was observed, the entire apparatus was disassembled, washed, re-assembled, re-autoclaved, and re-inoculated.

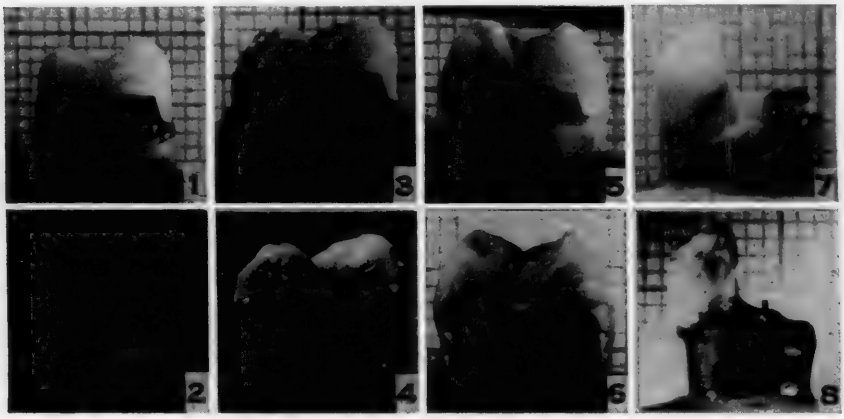

Fig. 2.—Examples of action of microorganisms on cut teeth in the artificial mouth. Upper photographs represent the original conditions and the lower photographs the conditions after 25 weeks. (1-2), *M. aureus* on Tooth 17; (3-4), *M. albus* on Tooth 18; (5-6), *Cl. perfringens* on Tooth 16; (7-8), *L. casei* (Hadley) on Tooth 13.

RESULTS

The action of the microorganisms on the teeth was observed after 8 and 25 weeks of exposure in the artificial mouth. The teeth were examined at 20- to 30-fold magnification, and the changes were recorded by photographs. A summary of the examinations is given below, and some of the changes are shown in Fig. 2.

Action of *Lactobacillus casei* (Hadley strain) on Tooth No. 13 (Fig. 2). This organism exhibited an unusually marked ability for decalcification, particularly of the intact enamel surface. The rate of dentinal matrix destruction was considerably slower

than the decalcification. As a result, much decalcified dentin was always present, and after 6 months most of the remaining dentin was spongy, decalcified, and very dark brown in color. Exposed cementum seemed to be destroyed.

Action of *Lactobacillus casei* (Mahler strain) on Tooth No. 12. This strain caused more dentin destruction than enamel loss, and cavitation under enamel occurred. Surface enamel, except in the cervical region, was fairly resistant to this organism. Decalcification of dentin proceeded slightly faster than matrix destruction; the dentin was brown in color. Exposed cementum seemed to be destroyed.

Action of *Streptococcus salivarius* (Niven strain) on Tooth No. 14. This organism exhibited only slight ability to attack the external enamel surface, except in the cervical region. Dentin decalcification proceeded somewhat faster than matrix destruction. The remaining dentin was deep brown in color. Exposed cementum seemed to be destroyed.

Action of *Streptococcus salivarius* (Kraus strain) on Tooth No. 20. The external enamel surface showed extensive signs of whitening and softening. The rate of the dentinal matrix attack was at least as rapid as decalcification, and no decalcified matrix remained. The remaining dentin was light yellow in color. Dentinal attack was much faster than that on enamel, as evidenced by extensive cavitation under the enamel. Cut enamel, particularly at the dentinoenamel junction, was rapidly attacked. Exposed cementum was destroyed.

Action of *Streptococcus faecalis* on Tooth No. 15. The organism attacked the external enamel surfaces only slightly, if at all. Dentinal attack was faster than enamel attack, and cavitation under the enamel occurred. The rate of decalcification of dentin was not faster than that of matrix destruction. The remaining dentin was slightly brown in color. Exposed cementum was destroyed.

Action of *Micrococcus aureus* on Tooth No. 17 (Fig. 2). Although the organism caused a slight softening of the external enamel surface, its main effect was on dentin. Much dentin was destroyed, and the matrix destruction occurred as rapidly as decalcification. Undermining of enamel resulted. The remaining dentin was slightly browner than originally. Exposed cementum was destroyed.

Action of *Micrococcus albus* on Tooth No. 18 (Fig. 2). The organism showed a rapid destruction of dentin and a slight but definite attack on the external enamel surface. Appreciable undermining of the enamel occurred. The residual dentin was somewhat decalcified and was brown in color; decalcification of dentin was somewhat faster than matrix destruction. Exposed cementum was destroyed.

Action of *Clostridium perfringens* on Tooth No. 16 (Fig. 2). Only a very slight decalcifying ability was observed for this organism. A limited but definite general surface destruction of dentin and an undercutting of enamel was noticeable. Decalcified dentinal matrix was rapidly destroyed. No pigmentation was produced. The exposed cementum was destroyed.

Action of *Neisseria catarrhalis* on Tooth No. 5. Over the period of the experiment, practically no action on the tooth could be observed. Two slight pits in the surface of the cut dentin developed, and these showed a slight softening on their surface.

A comparison was made of the ability of the microorganisms to produce acids under the conditions of the operations of the artificial mouth. The original medium had a pH of 6.2. Two types of measurements were made. In the first, indicated as pH_S, the pH of the medium was measured after it had been kept static in contact with the tooth for 24 hours. In the other method, the pH of the constantly dripping medium was measured on samples taken directly from the exit tube; these values are indicated as pH_D, and are greatly influenced by the rate of flow. The average values of 3 separate measurements are given in Table I.

TABLE I
RELATIVE ACIDOGENIC PROPERTIES OF MICROORGANISMS

ORGANISM	pH_S	pH_D
L. casei (Hadley)	4.0	4.9
L. casei (Mahler)	3.9	4.4
S. salivarius (Niven)	4.1	4.7
S. salivarius (Kraus)	4.2	6.3
S. faecalis	3.9	5.0
C. perfringens	4.8	5.9
M. aureus	4.8	5.7
M. albus	4.7	5.3

pH_S: Average pH of medium in contact with teeth for 24 hours.
pH_D: Average pH of medium from drip tube; highly influenced by drip rate.

DISCUSSION

In the present work, 8 pure strains of microorganisms of species that have been found in the oral cavity were shown to produce extensive destruction of teeth under in vitro conditions over a period of 25 weeks. The changes were quite apparent at 8 weeks. These species included 2 strains of *L. casei*, 2 of *S. salivarius*, and 1 each of *S. faecalis*, *M. albus*, *M. aureus*, and *C. perfringens*. One organism, *N. catarrhalis*, had no action.

The attack on the different tooth tissues varied considerably with the different organisms. These differences are shown in Table II and Fig. 2. Three types of attack were observed: action on the original enamel surface, decalcification of subsurface enamel and of dentin, and destruction of dentinal matrix. All of the active microorganisms seemed to affect the cementum.

TABLE II
SUMMARY OF ACTION OF MICROORGANISMS ON TOOTH SUBSTANCES DURING 25 WEEKS AT 36° C.

ORGANISM	DECALCIFIER		DESTROYER OF DECALCIFIED DENTINAL MATRIX	COLOR PRODUCED IN DENTIN
	ENAMEL SURFACE	DENTIN AND SUBSURFACE ENAMEL		
L. casei (Hadley)	Strong	Strong	Medium	Strong*
L. casei (Mahler)	Weak	Strong	Weak	Strong
S. salivarius (Niven)	Medium	Strong	Medium	Medium
S. salivarius (Kraus)	Medium	Strong	Strong	Weak*
S. faecalis	Weak	Medium	Medium	Medium
C. perfringens	Negative	Negative (?)	Medium	Negative
M. albus	Medium	Medium	Strong	Weak
M. aureus	Weak	Medium	Medium	Medium
M. catarrhalis	Negative	Negative	Negative	Negative

*The enamel surface also was somewhat discolored.

The extreme types of action were particularly noticeable in *L. casei* (Hadley strain) and *C. perfringens* (Fig. 2). This *L. casei* strain caused considerable destruction of surface enamel and extensive decalcification of dentin; the decalcified dentinal matrix was secondarily destroyed. The *C. perfringens* had no observable ability to attack enamel, but some loss of dentin and particularly of cementum occurred.

The other active microorganisms exhibited types of attack intermediate between these extremes. As pointed out earlier by Miller,[21] some of these organisms attack dentin much faster than enamel. In such instances, undermining of the enamel often occurred (Fig. 3).

With the possible exception of the attack by *C. perfringens,* the attack on dentin always showed a decalcification rate faster or at most equal to the rate of matrix destruction. When the decalcification rate was definitely more rapid than the rate of the matrix destruction, a mass of decalcified dentin remained which was secondarily destroyed (Fig. 2). In comparison, no sign of decalcification was evident on the tooth exposed to *C. perfringens,* although the cementum, in particular, was extensively removed. Primary decalcification could have occurred, but probably would have been evident on the susceptible cut subsurface enamel.

A pigmentation of the dentin seemed to be associated with the presence of decalcified dentinal matrix (Table II). Microorganisms exhibiting a rapid destruction of dentin produced little coloration of the remaining, fairly sound dentin. Of 3 organisms exhibiting a definite attack on the upper two thirds of the original enamel surface, 2 produced some pigmentation of the enamel surface.

Fig. 3.—Undercutting of dentin and attack on subsurface enamel. Enlargement of cut enamel surface of Tooth 15, (1) showing attack on subsurface enamel, (2) undercutting of enamel at cemento-enamel junction, and (3) enlargement of Tooth 17 showing cavitation of dentin under enamel.

The surface enamel exhibited a markedly greater resistance to attack than the subsurface enamel (see Fig. 3). This difference was clearly demonstrated on those surfaces of enamel which were cut perpendicularly to the original surfaces. Although the neighboring external surface in some instances was virtually unchanged from its original condition, the cut surface was extensively softened. When undermining of dentin occurred, the enamel lying near the dentinoenamel junction was often highly softened. The existence of a resistant surface layer of the enamel has been noted by numerous previous investigators,[5, 8, 22, 23, 25, 28] but was an especially prominent feature of the present work. Most of the organisms exhibited some ability to destroy cervical enamel beginning at the cemento-enamel junction, sometimes with undermining (Fig. 3). Previous studies in the artificial mouth demonstrated the marked susceptibility of this area of teeth to attack.[30]

The teeth used in the present study were all unerupted or partially erupted third molars. Some of the differences in types of attack shown by the various species of microorganisms, particularly on the external enamel, could have arisen from differences in the tooth structures. To some extent this difficulty was minimized, since 2 independent sets of experiments with similar results were carried out for most of the microorganisms. Miller[21-23] and other

workers[5, 9, 12, 26, 32, 34] have reported that teeth show considerable variability in their ability to withstand attack of microorganisms or acids. The use of older teeth might have greatly increased the differences in behavior of the various tooth tissues, especially the surface enamel.

The concentration of glucose present in the medium was arbitrarily established on the basis of earlier results[32] that 0.25 per cent glucose in the medium will allow both decalcification and matrix destruction to take place when a mixed culture from human saliva is allowed to grow on the teeth. The requirements for the individual pure strains were not investigated in the present work. Possibly other materials such as sucrose could also replace the glucose. The lack of attack of many organisms on intact dentin, such as that observed by Burnett and Scherp,[4] very likely arose from the absence of added sugars.

The actual degree of correspondence of the conditions used in this work with essential actual oral conditions is undetermined. The factors causing attack and localized lesions certainly agree well with those usually assumed as important in the human mouth.[10] The histologic description of lesions is also similar in most respects to that of natural lesions.[33]

In agreement with earlier investigations,[3, 5, 7, 11, 13-16, 19, 20] the present work indicates strongly that many types of oral microorganisms have the ability to destroy the tooth substances in the absence of lactobacilli and under conditions which might occur in the human mouth. A restriction of the bacterial factor in dental caries to lactobacilli species seems unwarranted, and necessitates a return to the view of earlier workers that numerous oral microorganisms are involved.

Fewer organisms appear to be able to attack the external enamel surface than can destroy subsurface enamel and dentin. Since complete enamel loss can occur solely by decalcification procedures,[30] no necessity for enamel matrix destruction exists, but the possibility of matrix destruction by some organisms is not excluded. Four microorganisms exhibited an ability to initiate attack on external enamel surfaces. One *Lactobacillus casei* strain showed the most pronounced effect of this type, but the other *L. casei* strain caused attack only at the cervical region. On the other hand, 2 strains of streptococci and the *M. albus* showed a definite action on the enamel surface. Because of the importance of the initiation of attack on enamel surfaces in relation to the etiology of dental caries, these results require additional study.

Caries of the dentin, especially of the undermining type, may result from the actions of other microorganisms which are only slow destroyers of enamel. As shown earlier by Miller,[21] and Pigman, Hawkins, West, and Gaston,[30] complete destruction of dentin required both decalcification and matrix destruction. Symbiotic relations of the microorganisms may increase the individual effects.

The previous evidence of the correlation of lactobacilli counts with caries incidence may well represent a secondary relationship. As indicated in our earlier work,[32] this organism appears to have a definite requirement for sugars. Jay[17] has expressed the view that its appearance in the oral cavity may signify the existence of sufficient nutrients (probably sugars) to allow decalcification

processes to proceed. However, as indicated in the present work and earlier by Sullivan[36] and others, lactobacilli, even of the same accepted species, differ markedly in decalcification characteristics. The actual decalcification may result from the combined actions of some of the numerous other oral microorganisms, present in large amounts.

SUMMARY

Eight strains of microorganisms of the type occurring in the mouth were found to destroy human teeth extensively in less than 25 weeks, when subjected to the conditions of the artificial mouth. These organisms were *Lactobacillus casei* (2 strains), *Streptococcus salivarius* (2 strains), *Streptococcus faecalis, Micrococcus pyogenes* var. *aureus, Micrococcus pyogenes* var. *albus,* and *Clostridium perfringens.* One other microorganism, *Neisseria catarrhalis,* was tested and found to be without action.

These microorganisms exhibited marked differences in their ability to attack the various tissues of teeth. One strain of *L. casei* exhibited intensive decalcifying action; it had only a moderate ability to destroy decalcified dentinal matrix, but it destroyed external enamel surfaces readily. The other extreme of action was shown by *C. perfringens,* which destroyed calcified and decalcified dentinal matrix, without any obvious signs of decalcification. Other organisms showed actions intermediate between the extremes. Some destroyed dentin more rapidly than enamel and produced undercutting of enamel. A pigmentation of dentin seemed to be associated with a rate of dentinal decalcification greater than that of matrix destruction.

A restriction of the bacterial factor in dental caries to lactobacilli species seems an unwarranted oversimplification, but lactobacilli may be "indicator" microorganisms useful for showing the existence of conditions which permit rapid decalcification. The observed ability of a number of oral bacteria to destroy teeth rapidly, under in vitro conditions, suggests that many types of bacteria may participate in the carious process. The variable action of the several types of microorganisms on the different tissues of teeth indicates that carious lesions in different anatomical locations may have diverse etiologies, arising from the actions of many types of microorganisms acting singly, jointly, or consecutively.

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Erratum

Abstract 29 of the Scientific Proceedings of the British Division of the International Association for Dental Research, which appeared on page 966 of the December JOURNAL, is reprinted here. Please note that line 7 has been corrected.

29. THE CARBOHYDRATE CONTENT OF HUMAN DENTAL ENAMEL. *M. V. Stack, M.R.C. (External) Staff, Dental School, University of Bristol.* Levels of carbohydrate in the outermost region of thoroughly cleaned enamel were found to be at least 10 times those of the main body of enamel. This layer was ground off in order to determine the range in carbohydrate content of representative enamel. Samples were prepared by flotation from 30 permanent and 20 deciduous teeth, using 1 tooth from each patient. Comparable values for total carbohydrate, which reacted as glucose, were shown by analyses with solutions or orcinol or of anthrone in 70 per cent sulphuric acid. The mean value for permanent teeth, 15 ± 5 (S.D.) mg. per cent, was not significantly different from that for deciduous teeth (16 ± 5 mg. per cent). Enamel from permanent molars was found to contain two-thirds as much carbohydrate as that from other types of permanent teeth. This observation was supported by tests on pairs of teeth from 5 patients, and was thought to depend on the smaller surface:volume ratio of molar teeth, and on the greater proportion of carbohydrate in the outer region of the enamel. Correlations were not observed between carbohydrate levels and age or caries experience. The carbohydrate content of the enamel from 5 pairs of teeth from 1 individual was 15 ± 2 mg. per cent. Samples of developing enamel in which the organic contents were 10, 20, and 40 times the level in mature enamel were found to contain 7, 10, and 13 times as much carbohydrate, respectively. Pooled "chalky" enamel, in which had been found 3 to 4 times as much organic matter as normal enamel (Stack, *Brit. D. J.* **96**: 73, 1954), showed a carbohydrate level 12 times greater than normal.

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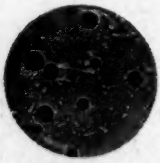

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